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Green tea catechins potentiate the effect of antibiotics and modulate adherence and gene expression in *Porphyromonas gingivalis*

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ABSTRACT

Objectives: A number of studies have brought evidence that green tea catechins may contribute to periodontal health. The objective of this study was to investigate the ability of a green tea extract and its principal constituent epigallocatechin-3-gallate (EGCG) to potentiate the antibacterial effects of antibiotics (metronidazole, tetracycline) against *Porphyromonas gingivalis*, and to modulate the adherence to oral epithelial cells and expression of genes coding for virulence factors and the high temperature requirement A (HtrA) stress protein in *P. gingivalis*.
Methods: A broth microdilution assay was used to determine the antibacterial activity of the green tea extract and EGCG. The synergistic effects of either compounds in association with metronidazole or tetracycline were evaluated using the checkerboard technique. A fluorescent assay was used to determine bacterial adherence to oral epithelial cells. The modulation of gene expression in *P. gingivalis* was evaluated by quantitative RT-PCR. The *Vibrio harveyi* bioassay was used for monitoring quorum sensing inhibitory activity.
Results: The MIC values of the green tea extract on *P. gingivalis* ranged from 250 to 1000 μg/ml, while those of EGCG ranged from 125 to 500 μg/ml. A marked synergistic effect on *P. gingivalis* growth was observed for the green tea extract or EGCG in combination with metronidazole. Both the green tea extract and EGCG caused a dose-dependent inhibition of *P. gingivalis* adherence to oral epithelial cells. On the one hand, green tea extract and EGCG dose-dependently inhibited the expression of several *P. gingivalis* genes involved in host colonization (*fimA*, *hagA*, *hagB*), tissue destruction (*rgpA*, *kgp*), and heme acquisition (*hem*). On the other hand, both compounds increased the expression of the stress protein *htrA* gene. The ability of the green tea extract and EGCG to inhibit quorum sensing may contribute to the modulation of gene expression.
Conclusions: This study explored the preventive and therapeutic potential of green tea catechins against periodontal disease. In addition to inhibit growth and adherence of *P. gingivalis*, a green tea extract and its main constituent EGCG was found to decrease the expression of genes coding for the major virulence factors.

1. Introduction

Periodontitis is defined as a progressive inflammatory disease affecting the soft and hard tooth-supporting tissues, including the gingiva, periodontal ligament, and alveolar bone (Pihlstrom, Michalowicz, & Johnson, 2005). Severe periodontitis that may cause loosening and subsequent loss of the affected teeth, affects approximately 8.5% of the adult population in the United States (Eke, Dye, Wei, Thornton-Evans, & Genco, 2012). Over the last decade, evidence has accumulated to suggest that periodontal disease represents a risk factor for systemic complications such as cardiovascular disease, type 2 diabetes, pneumonia, and preterm low birth weight (Otomo-Corgel, Pucher, Rethman, & Reynolds, 2012). The transition from health to periodontal disease occurs when the equilibrium of the oral microbial ecosystem is broken in favor of a limited number of Gram negative anaerobic bacteria, known as periodontopathogens that most individuals harbor in low concentrations in their subgingival sites (Bartold & Van Dyke, 2013; Berezow & Darveau, 2011). Although periodontopathogens

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are essential to initiate the disease, periodontal tissue and bone destruction is mostly a consequence of the uncontrolled host immune response to the continuous bacterial aggression (Ebersole et al., 2013; Liu, Lerner, & Teng, 2010). More specifically, periodontopathogens and their products (i) trigger resident and immigrant host cells to express tissue degrading enzymes, and (ii) provoke an immune response that results in the release of high amounts of cytokines by lymphocytes, macrophages, and other cell lineages. These cytokines can subsequently activate several host degradative pathways and ultimately mediate bone resorption (Ebersole et al., 2013; Liu et al., 2010).

Strong evidence has accumulated to implicate *Porphyromonas gingivalis*, a Gram-negative anaerobic bacterium, as one of the key pathogens in the chronic form of periodontitis (Berezow & Darveau, 2011; Socransky, Haffajee, Cugini, Smith, & Kent, 1998). *P. gingivalis*, which can be detected in as much as 85% of the diseased sites (Yang, Huang, & Chou, 2004), expresses a large array of virulence factors that allow the bacterium to colonize the subgingival sites, to circumvent the immune system and to cause tissue destruction (Bostanci & Belibasakis, 2012). While the major role of type I fimbriae (FimA) of *P. gingivalis* is to mediate biofilm formation, adherence to saliva-coated surfaces, and adherence to gingival epithelial cells, it can also trigger an inflammatory response (Andrian, Grenier, & Rouabhia, 2006; Enersen, Nakano, & Amano, 2013). Hemagglutinins also contribute to the establishment of *P. gingivalis* in subgingival sites via binding to oligosaccharide receptors present on human cells (Du, Pellen-Mussi, Chandad, Mouton, & Bonnaure-Mallet, 1997). Gingipains, which are cysteine proteinases, are responsible for most of the extracellular and cell-bound proteolytic activities produced by *P. gingivalis* (Fitzpatrick, Wijeyewickrema, & Pike, 2009; Kadowaki et al., 2000). Data analysis suggests that three different genes code for the gingipains: Arg-gingipain A (*rgpA*), Arg-gingipain B (*rgpB*) and Lys-gingipain (*kgp*) (Fitzpatrick et al., 2009; Kadowaki et al., 2000). RgpA and Kgp, in addition to possess a catalytic domain, also have an hemagglutinin/adhesin domain responsible for both hemagglutinating activity and specific binding to host proteins, including fibrinogen, fibronectin, and laminin (Fitzpatrick et al., 2009; Kadowaki et al., 2000). Moreover, both Arg- and Lys-gingipains can trigger a pro-inflammatory response in macrophages resulting in the secretion of tumor necrosis factor-α (TNF-α) and interleukin-8 (IL-8) (Grenier & Tanabe, 2010). Finally, *P. gingivalis* is known to produce an hemolysin that lyses erythrocytes, thereby releasing hemoglobin which provides heme required for growth of the bacterium (Deshpande & Khan, 1999)

Bacteria have developed various mechanisms to survive stressful conditions. More specifically, under stress, the periplasmic high temperature requirement A (HtrA) protein acts both as a serine protease to degrade unfolded, misfolded or otherwise proteins or as a chaperone protein to protect protein structure (Skorko-Glonek et al., 2003). In *P. gingivalis*, HtrA has been reported to be responsible for resistance to oxidative stress (Yuan, Rodrigues, Belanger, Dunn, & Progulske-Fox, 2008). In addition, using a *htrA*-deficient mutant, evidence was obtained that this stress protein may be important for *P. gingivalis* virulence and survival in in vivo animal models (Yuan et al., 2008). This may be related to an increased susceptibility of bacteria to environmental stresses they encounter in the host.

Green tea, an aqueous aromatic infusion of steamed and dried non-fermented leaves of the plant *Camellia sinensis*, has a high catechin content (flavan-3-ols), including epigallocatechin-3-gallate (EGCG) that represents about 59% of total catechins (Cabrera, Artacho, & Giménez, 2006). Besides the catechins, green tea also contains gallic acid, phenolic acids such as chlorogenic and caffeic acids, and flavonols, including myricetin and quercetin (Cabrera et al., 2006). Tea is considered as one of the most important functional food since in addition to fulfill basic nutrition requirements it provides physiological benefits. Indeed, human, animal, and in vitro studies have brought evidence that green tea catechins may contribute to reducing the risk and/or severity of many systemic conditions and diseases, such as diabetes, cardiovascular disease and some forms of cancer (Cabrera et al., 2006; Cooper, 2012; Da Silva Pinta, 2013). The beneficial properties of green tea catechins, more specifically EGCG, have been mostly associated to their antioxidative, anti-inflammatory, antimicrobial and anti-carcinogenic properties (Cabrera et al., 2006; Cooper, 2012; Da Silva Pinta, 2013). While oral health benefits have been attributed to green tea throughout history, only a limited number of scientific studies to validate these benefits have been performed (Gaur & Agnihotri, 2014; Koyama et al., 2010; Kushiyama, Shimazaki, Murakami, & Yamashita, 2009; Narotzki, Reznick, Aizenbud, & Levy, 2012). An epidemiological study on periodontal diseases published in 2009 showed that drinking one or more cups of green tea a day was associated with a reduction, although modest, of the incidence and severity of periodontal disease (Kushiyama et al., 2009). Moreover, clinical studies have shown that local delivery (gel, chip) of green tea catechins in diseased periodontal pockets enhances the efficacy of the conventional periodontal treatment consisting in scaling and root planning (Kudva, Tabasum, & Shekhawat, 2011; Hirasawa, Takada, Makimur, & Otake, 2002). Despite the above studies showing that green tea catechins have a positive impact on periodontal health, additional work is required to determine how these polyphenols exert their beneficial effects. In this study, we hypothesized that a green tea extract and its principal constituent EGCG modulate the adherence and expression of genes coding for virulence factors and the HtrA stress protein in *P. gingivalis*. Moreover, we also tested whether green tea polyphenols may potentiate the antibacterial effects of antibiotics (metronidazole, tetracycline) against *P. gingivalis*.

2. Materials and methods

2.1. Green tea extract and EGCG

The commercial green tea extract (Hangzhou Gosun Biotechnologies Co., Ltd., Hangzhou Zhejiang, China) used in this study had a polyphenol content of 98.42%, including 47.92% EGCG, according to the company's data sheet. Table 1 reports the detailed catechin content of the green tea extract. A stock solution was freshly prepared by dissolving 20 mg of powder in one ml of sterile warm distilled water and filtering the solution through a 0.22-μm pore size membrane filter. EGCG (Sigma–Aldrich Canada Ltd., Oakville, ON, Canada), the predominant catechin in the green tea extract, was also dissolved in sterile distilled water at a concentration of 10 mg/ml and was sterilized by filtration as above.

Table 1
Catechin content of the green tea extract used in the study.

Component	% (w/w)
Total polyphenols	98.42
Total catechins	82.6
EGCG	47.92
Epigallocatechin	7.56
DL-Catechins	2.16
Epicatechin	6.19
Gallate catechin gallate	4.54
Epicatechin gallate	14.23