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Research paper

Regulation of matrix metalloproteinase secretion by green tea catechins in a three-dimensional co-culture model of macrophages and gingival fibroblasts

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ABSTRACT

Objectives: Elevated levels of matrix metalloproteinases (MMPs) have been associated with the active phases of tissue and bone destruction in periodontitis, an inflammatory disease characterized by a significant breakdown of tooth support. In the present study, we used a three-dimensional (3D) co-culture model of macrophages and gingival fibroblasts to investigate the ability of a green tea extract and its major constituent epigallocatechin-3-gallate (EGCG) to regulate the secretion of MMP-3, -8, and -9.
Methods: The 3D co-culture model was composed of gingival fibroblasts embedded in a type I collagen matrix overlaid with macrophages. Two arbitrary ratios were tested. The ratio composed of 1 macrophage to 10 fibroblasts was used to mimic a slightly inflamed periodontal site while the ratio composed of 10 macrophages to 1 fibroblast was used to mimic a severely inflamed periodontal site. The 3D co-culture model was pre-treated for 2 h with either the green tea extract or EGCG. It was then stimulated with *Aggregatibacter actinomycetemcomitans* lipopolysaccharide (LPS). The model was also first stimulated with LPS for 2 h and then incubated with the green tea extract or EGCG. The concentrations of secreted MMP-3, -8, and -9 were quantified by enzyme-linked immunoassays.
Results: When the 3D co-culture model was stimulated with *A. actinomycetemcomitans* LPS, the 10:1 ratio of macrophages to gingival fibroblasts was associated with a highest secretion of MMP-3 and -9 and, to a lesser extent, MMP-8, than the 1:10 ratio. Non-cytotoxic concentrations of the green tea extract or EGCG reduced the basal secretion levels of all three MMPs. A 2-h treatment with the green tea extract or EGCG prior to the stimulation with LPS resulted in a dose-dependent decrease in MMP secretion, with MMP-9 showing the most significant decrease. A decrease in MMP secretion was also observed when the green tea extract or EGCG was added following a 2-h stimulation with LPS.
Conclusions: Our results suggested that green tea catechins, and more specifically EGCG, offer promising prospects for the development of a novel adjunctive treatment for periodontitis because of their ability to decrease the secretion of MMPs, which are important tissue-destructive enzymes produced by mucosal and immune cells.

1. Introduction

Periodontitis is a multifactorial chronic inflammatory disease of bacterial origin that causes the destruction of the tooth-supporting tissues, including the periodontal ligament and alveolar bone (Loesche & Grossman, 2001). Depending on the age group, up to 15% of the population is affected by severe forms of this disease (Albandar, 2011; Eke, Dye, Wei, Thornton-Evans, & Genco, 2012; Kassebaum et al., 2014) that, if left untreated, may result in tooth loss and systemic complications such as cardiovascular diseases, diabetes mellitus, and preterm birth and/or low birth weight (Chambrone, Guglielmetti, Pannuti, & Chambrone, 2011; Chapple & Genco, 2013; Teles & Wang, 2011). A limited number of Gram-negative bacteria that colonize subgingival sites and activate the host immune response have been associated with periodontitis (Socransky & Haffajee, 2005). *Aggregatibacter actinomycetemcomitans* is a key etiological agent of aggressive periodontitis, which is characterized by a rapid loss of clinical attachment and alveolar bone and which usually affects young adults (Nibali, 2015; Slots & Ting, 1999).

Fibroblasts are a major constituent of gingival connective tissue (Hassell, 1993). These mucosal cells produce structural connective tissue proteins, glycoproteins, and glycosaminoglycans, as well as

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Table 1
Secretion of MMP-3, MMP-8, and MMP-9 by the 3D co-culture model with the 1:10 macrophage:fibroblast ratio or 10:1 macrophage:fibroblast ratio stimulated or not with *A. actinomycetemcomitans* LPS.

MMP	Amount of MMP secreted (pg/mL)			
	1:10 ratio		10:1 ratio	
	No LPS	+ LPS	No LPS	+ LPS
MMP-3	7,401 ± 752	12,269 ± 1,928*	21,462 ± 242	30,365 ± 1,852*
MMP-8	32 ± 4	55 ± 7*	465 ± 27	648 ± 31*
MMP-9	1,522 ± 79	2,637 ± 237*	128,477 ± 30,635	183,473 ± 4,509*

* $p < 0.05$:significantly different from the control cells without LPS stimulation.

immunoregulatory cytokines and chemical mediators that may modulate the chronic inflammation associated with periodontitis (Hassell, 1993; Takashiba, Naruishi, & Murayama, 2003). Monocytes and macrophages, which are found in higher numbers in active periodontal lesions than in inactive sites (Zappa, Reinking-Zappa, Graf, & Espeland, 1991), are key members of the innate immune system and play a critical role in the host response during chronic infections such as periodontitis (Hasturk, Kantarcy, & Van Dyke, 2012). These immune cells are found mostly in gingival connective tissues and are also a major part of the inflammatory infiltrate (Hassell, 1993; Schroeder & Listgarten, 1997). The multifunctional roles of gingival fibroblasts and monocytes/macrophages make them important players in the initiation and maintenance of inflammatory processes and the alveolar bone loss observed in periodontitis (Kornman, Page, & Tonetti, 1997). Monocytes/macrophages and fibroblasts produce large amounts of matrix metalloproteinases (MMPs), which are a family of zinc-containing and calcium-dependent endopeptidases secreted or released as zymogens that can be converted into their active forms by proteolytic cleavage or by oxidative components (Nagase, 1997). These host-derived proteolytic enzymes are involved in the remodelling of the extracellular matrix and basement membrane during various physiological and pathological processes (Uitto, Overall, & McCulloch, 2003). During periodontitis, the continuous high secretion of MMPs by host cells following stimulation by periodontopathogens contributes to periodontal tissue destruction (Ejeil et al., 2003; Makela, Salo, Uitto, & Larjava, 1994; Sorsa et al., 2006). This is supported by the fact that MMP levels and activity are significantly higher in the gingival tissue and gingival crevicular fluid of periodontitis subjects (Ejeil et al., 2003; Makela et al., 1994; Sorsa et al., 2006).

Natural bioactive compounds endowed with a capacity to modulate the host inflammatory response by interfering with cell signaling pathways have received considerable attention as promising agents for the treatment of periodontitis (Paquette and Williams, 2000; Souza, Rossa, Garlet, Nogueira, & Cirelli, 2012). Given the key roles played by MMPs in periodontal tissue destruction, natural compounds with the ability to down-regulate MMP production by host cells may be of therapeutic interest. Green tea (*Camellia sinensis*) catechins, especially epigallocatechin-

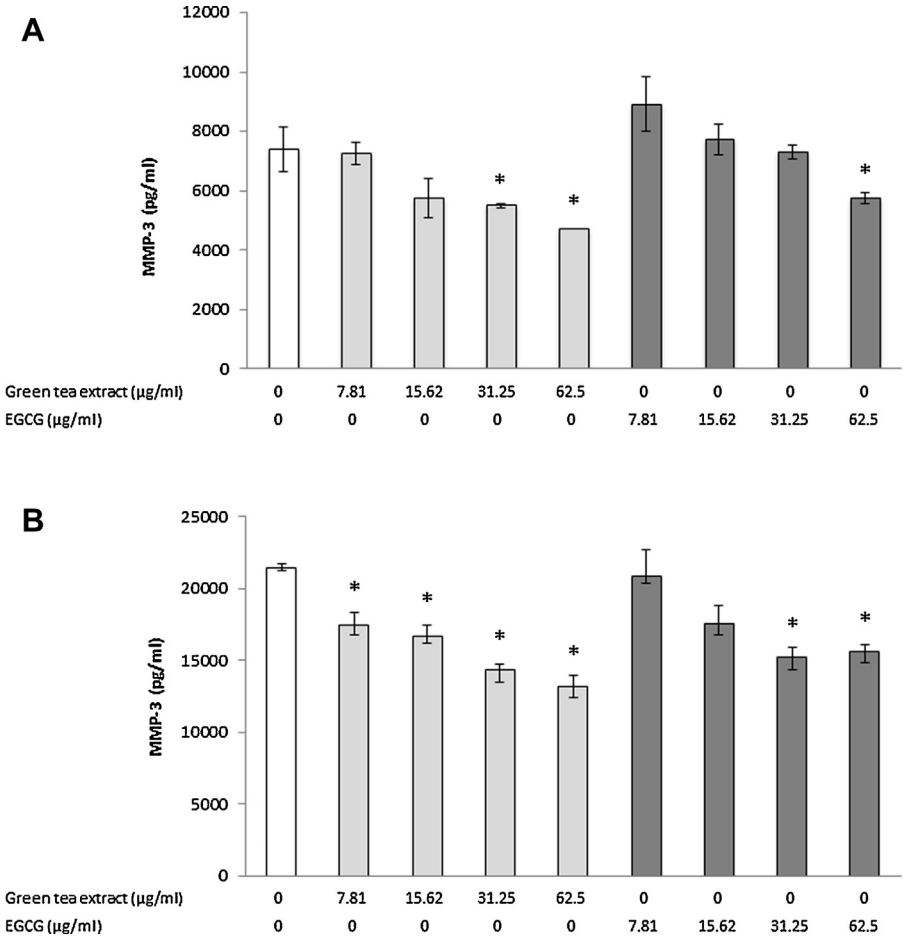

Fig. 1. Effects of the green tea extract and EGCG on the basal levels of MMP-3 secreted by the 3D co-culture model. Panel A: Ratio of 1 macrophage to 10 fibroblasts that mimics a slightly inflamed site. Panel B: Ratio of 10 macrophages to 1 fibroblast that mimics a severely inflamed site. *, $p < 0.05$: significantly different from control cells (without the green tea extract or EGCG).