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Profiling cytokine levels in chlorhexidine and EGCG-treated odontoblast-like cells

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ABSTRACT

Objective. To screen the effect of two compounds, chlorhexidine diacetate (CHX) and epigallocatechin-gallate (EGCG), on the levels of cytokines produced by odontoblast-like cells (MDPC-23).

Methods. Cells were seeded at 24 h and 48 h with serial dilution of the compounds to determine cell metabolic activity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay ($n = 3$). Cells with no compound treatment were used as control (Ctr). For the highest equal non-cytotoxic compound dilution tested at 48 h cell treatment, total protein concentration was measured using a Pierce bicinchoninic acid (BCA) assay ($n = 3$), and expression of 23 cytokines was analyzed using the Bio-Plex cytokine assay ($n = 2$). Data were analyzed by one-way ANOVA and Tukey's test ($\alpha = 5\%$).

Results. The MTT assay revealed that at 24 h and 48 h, CHX and EGCG did not reduce cell metabolic activity at concentrations of 2.5–20 μM (CHX) and 2.5–160 μM (EGCG), respectively ($p > 0.05$). At 48 h, total protein levels were consistent across all groups for 20 μM compound dilution (Ctr: 1.04 mg/mL; CHX: 0.98 mg/mL; and EGCG: 1.06 mg/mL). At 20 μM dilution, both CHX and EGCG significantly increased the secretion of IL-1β, IL-10, IL-12, KC, MIP-1α, IFN-γ and IL-6 ($p < 0.05$). Treatment with CHX significantly increased secretion of IL-4 and RANTES ($p < 0.05$). Treatment: with EGCG significantly increased Eotaxin secretion ($p < 0.05$). Both CHX and EGCG significantly decreased secretion of IL-17 ($p < 0.05$). GM-CSF and TNF-α did not present significant change in secretion after treatment with either CHX or EGCG ($p > 0.05$).

Significance. Both CHX and EGCG modulate secretion of various inflammatory and anti-inflammatory mediators in odontoblastic cells.

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1. Introduction

Cytokines are produced by different cells of the body and although their grouping into cytokine families is not consensual in the literature, it is accepted that they can be arranged into structural families that include: interleukins, chemokines, interferons, transforming growth and tumor necrosis factor families. Families of cytokines share sequence similarity and exhibit homology and some promiscuity in their reciprocal receptor systems. Despite being structurally related, members belonging to the same cytokine family may exhibit rather diverse functions. Essentially, cytokines are small (~5 to 20 kDa) signaling proteins synthesized by a range of both immune and tissue structural cells in response to different cellular stresses and stimuli. These molecules are able to regulate cellular immune and pro- and anti-inflammatory responses, and further modulate gene expression and biochemical responses in target cells via second messenger signaling mechanisms [1]. The fine-tuned cytokine networks maintain a balance between pro- and anti-inflammatory processes creating the desired environment for tissue repair [2]. While cytokines and chemokines are likely produced in response to bacterial invasion, interesting reports show molecules such as transforming growth factor-β (TGF-β), insulin-like growth factor-1 and -2 (IGF-1 and -2), fibroblast growth factor-2 (FGF-2) sequestrated within dentin [1]. These can be released by acids and restorative materials [3], and may stimulate, for instance, expression of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) by macrophages [4]. Both TNF-α and IL-1β can in turn induce expression of IL-8, a neutrophil recruiter and activator [5]. The interactions and clinical effects of cytokines and chemokines sequestrated within dentin and/or produced by odontoblasts, on tissue remodeling, certainly entail further understanding.

Clinical applications of chlorhexidine (CHX) in restorative dentistry have been long advocated. Studies have recommended the cleansing of dentin with CHX prior to pulp capping and the use of cavity liners. This would eliminate bacteria lodged in dentinal tubules after mechanical caries removal, encouraging pulp tissue repair by controlling bacterial contamination and inflammation [6]. Others have demonstrated that CHX is capable of arresting caries when applied to dentin [7]. It has also been shown that due to antiproteolytic activity against matrix metalloproteinases (*i.e.*, MMP-2, -8, and -9) [8] and cysteine cathepsins [9], the use of CHX on demineralized dentin prior to bonding agent application prevents the degradation of hybrid layer, maintaining the integrity of adhesive restorations over longer periods of time [10]. However, certain limitations of CHX such as its synthetic nature and relative (dose-dependent) cytotoxicity to odontoblastic cells [11,12], which may induce cell inflammation, has motivated researchers to investigate alternative compounds.

Some attention has been directed to the use of epigallocatechin-gallate (EGCG), a natural polyphenol derived from green tea, in dentin treatment. EGCG is an effective antimicrobial agent against *Streptococcus mutans* and inhibits acid production by caries pathogens in dental biofilm [13,14]. It has been reported that EGCG inhibits dentinal proteases [15], thus preserving the long-term dentin bond strength with equal effect to chlorhexidine [16]. Once released from restorative copolymers, EGCG retains antibacterial and antiproteolytic activities [17,18]. Natural polyphenols in general, including EGCG, are recognized to have anti-inflammatory properties and to present low cytotoxicity [19]. EGCG was also shown to attenuate production of the pro-inflammatory IL-12 in murine macrophages [20] and of TNF-α in arthritic joints of green tea polyphenols fed mice [21]. In addition, EGCG demonstrated a suppression of NF-κB activation leading to inhibition of pro-inflammatory cytokine release in bacteria-challenged dental pulp cells [22].

To our knowledge, the possible inflammatory or anti-inflammatory responses of CHX and EGCG on odontoblastic cell lines have never been investigated. Odontoblasts represent the first layer of cells to respond to dentinal stimuli and therapies, and therefore can initiate immunological responses through cytokine signaling. Given the wide range of use and clinical applications of both compounds, including dentinal regions with close proximity with pulp or with micro-exposure to pulp, it was the purpose of this study to profile the effect of CHX and EGCG treatment on the expression of pro-inflammatory and anti-inflammatory cytokines, chemokines, and growth factors on an odontoblast-like cell model. The hypothesis of this study is that the cytokinome of EGCG-treated odontoblast like cells would profile in a predominantly anti-inflammatory manner, whereas the chlorhexidine-treated cells, in a pro-inflammatory manner when compared to non-treated cells.

2. Material and methods

2.1. MDPC-23 odontoblast-like cell culture

The immortalized mouse dental papilla MDPC-23 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM; with high glucose, L-glutamine and sodium pyruvate) supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY, USA), with 100 IU/mL penicillin, 100 mg/mL streptomycin and 0.25 mg/mL fungizone (Gibco). Cells were sub-cultured every 2 days and allowed to grow in a humidified incubator at 37 °C with 5% CO_2 (Isotemp; Fisher Scientific, Pittsburgh, PA, USA).

2.2. MTT cytotoxicity assay

MDPC-23 odontoblast-like cells were seeded in DMEM in 96-well plate at 2×10^4 cells/200 μL/well and allowed to grow for 24 h. Afterwards, cells were seeded ($n = 3$) with serial dilution (from 2.5 μM to 160 μM) of either chlorhexidine diacetate (CHX) or epigallocatechin-gallate (EGCG) (Sigma–Aldrich, St. Louis, MO, USA) in DMEM for 48 h. Cells with no compound treatment were used as control (Ctr). Cells metabolic activity was evaluated by the MTT (3-(4,5-dimethylth-iazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (Roche Applied Science, Indianapolis, IN, USA) as previously described [18]. Data were analyzed by one-way ANOVA and Tukey post hoc test ($\alpha = 0.05$). For the following assays, the highest and equal dilution of compounds that did not induce cytotoxicity was used.

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