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ORIGINAL ARTICLE

Anti-inflammatory effects of bamboo salt and sodium fluoride in human gingival fibroblasts—An *in vitro* study

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Abstract Dental caries preventive agents, such as sodium fluoride (NaF) and bamboo salt (BS), are known to cause cellular growth that is characterized by morphological and gene expression changes. This study was designed to investigate the dual effect of NaF and BS on interleukin (IL)-1β-induced gingival inflammation. Under *in vitro* experimental conditions, exposure to a subcytotoxic dose of IL-1β enhanced human gingival fibroblast inflammation, as characterized by increased levels of inflammation-associated proteins. A combination of NaF and BS significantly protected fibroblasts from IL-1β-induced cellular deterioration. Exposure to NaF and BS induced the cell growth and no changes in viability were found with the Lactate Dehydrogenase Assay (LDH) assay at the NaF and BS concentration analyzed. Molecular analysis demonstrated that NaF and BS increased resistance to inflammation by reduction of IL-1β, IL-8, and tumor necrosis factor (TNF)-α production. In addition, NaF and BS decreased the expression of IL-1β, IL-8, and TNF-α mRNA in IL-1β-induced human gingival fibroblast cells. The study identifies a new role for NaF and BS in the IL-1β-induced inflammation of gingival fibroblasts and provides a potential target for gingival protection.

Conflicts of interest: All authors declare no conflicts of interest.

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Introduction

Sodium fluoride (NaF) has long been recognized as one of the best public health measures in the prevention of dental caries [1]. It also has a potential role in the prevention of osteoporosis [2]. Fluoride is present in natural fresh water and a concentration of 1.6—1.8 ppm in drinking water is the threshold for the risk of dental fluorosis in the population [3]. The concentration of fluoride that cells are exposed to appears to be a critical factor in determining any changes in behavior that may occur. Li et al used organ culture to show that 2 mM fluoride affected the Rho/Rho-associated protein kinase (ROCK) signal transduction pathway, resulting in elevated F-actin in ameloblasts [4]. Millimolar levels of fluoride were also found to induce endoplasmic reticulum stress, apoptosis, and caspase-mediated DNA fragmentation in enamel organ epithelial-derived cell lines [5]. It was reported that the toxicity on a Smulow-Glickman human gingival epithelial cell line was first observed at an exposure to 1 mM NaF for 24 hours [6]. Thaweboon et al [7] reported a biphasic effect of fluoride at mM fluoride levels in dental pulp cells. Lower concentrations of fluoride promoted the proliferation of cells of the epithelial ameloblast-lineage, with the peak ~16 μM NaF [8]. Although the sensitivity to fluoride differs with the kind of cell and donor's age, it is true that higher mM fluoride levels are toxic to human cells.

In Korea, bamboo salts (BS) are mainly ingested for health reasons because they are produced using processes that lead to decreased toxicity and the conversion of acidity to strong alkalinity when compared to sun-dried salts. BS is known to have therapeutic effects for diseases such as viral diseases, dental plaque, and inflammatory disorders [9—11]. The remineralization effects of bamboo salt on incipient artificial enamel caries were also reported [12].

Gingiva is covered by stratified squamous epithelium with architectural characteristics unique to dental areas. Several cell types have been identified within gingival connective tissue and gingival fibroblasts, which account for most connective tissue cells, and are likely to be responsible for the constant functional adaptation of gingival connective tissue [13]. Gingival fibroblasts play a major role in normal connective tissue turnover, as well as in wound healing repair and regeneration [14]. Gingival inflammation is a chronic disorder characterized by the breakdown of tooth-supporting tissues, and producing a loss of dentition. The cause is an ecological imbalance between the microbial biofilm on teeth and an impaired host inflammatory response. The disease involves the breakdown of the gingival connective tissue, namely gingival fibroblast dysfunction. Inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-8, are rapidly induced and expressed in inflammation in an antigen-independent manner. TNF-α is a pleiotropic cytokine capable of altering physiological and immunological sequelae as well as mediating the pathophysiological responses of various disease conditions [15]. IL-1β is a potent multifunctional proinfiammatory polypeptide produced by monocytes and tissue macrophages [16]. IL-1β attracts and activates immune cells and controls the expression of most immunomodulatory genes [17]. It is well established that IL-1β is a major cytokine involved in the infiammatory process in periodontitis [18]. IL-1β acts directly on local fibroblasts in infiammatory conditions, inducing a variety of genes and helping to create an activated phenotype characterized by hyperplasia and invasiveness [19].

Evidence has been found indicating the role of low concentration NaF in proliferation of human gingival cells. However, to date no studies have been carried out to determine its status and mechanism of action during inflammation. Thus, it is important to examine the role of BS and NaF in the pathophysiology of gingival inflammation. The purpose of the present study was to investigate the effect of a combination of BS and NaF on inflammatory cytokine production and expression in human recombinant IL-1β-stimulated gingival inflammation.

Materials and methods

Cell culture

Human gingival fibroblast (HGF) was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS). They were seeded in 60 mm plastic tissue culture dishes and incubated in 5% CO_2 at 37°C. When the cells reached subconfluence, they were harvested and subcultured.

Cell proliferation

Cultured cells were harvested from 80% confluent monolayer cultures by a brief trypsinization with 0.1% trypsin and 0.1% EDTA. The cells were seeded at a density of 5000 cells per well of 96-well tissue culture plates and cultured for 24 hours in DMEM. The medium was replaced with serum-free medium for 18 hours and then treated with DMEM supplemented with BS and NaF. Cell proliferation was assessed using a MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetraz olium) assay at 24 hours after treatment. CellTiter 96 AQueous One Solution reagent (Promega, Madison, WI, USA) was added directly to each well and incubated for 2 hours, the absorbance at 490 nm was then measured using a microplate reader (iMark, Bio-Rad, Hercules, CA, USA).

Cytotoxicity assay

To determine the cytotoxic activity of BS and NaF, the Cytotox 96 nonradioactive cytotoxicity assay (Promega Corporation, Madison, WI, USA) was used. Briefly, the cells were incubated with BS and NaF for 24 hours, and then 50 μL of supernatant was assayed for LDH activity following the manufacturer's protocol. All of the conditions were assayed in triplicate. Controls for spontaneous LDH release in experimental cells, as well as maximum release, were prepared. The calculation of cytotoxicity percentage was carried out as follows:

$$\text{cytotoxicity}\ (\%) = (\text{experimental LDH release} / \text{maximum LDH release}) \times 100. \quad (1)$$