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ORIGINAL ARTICLE

Effects of arecoline on cell growth, migration, and differentiation in cementoblasts

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arecoline;
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Abstract *Background/purpose:* Studies have supported a higher prevalence of periodontal disease among areca quid chewers than non-chewers. However, few studies have stated the effects of areca quid on periodontal tissues. The aim of this study was to investigate the inhibitory effects of arecoline, the major alkaloid of areca nut, on murine immortalized cementoblast cell line (OCCM.30).
Materials and methods: Cytotoxicity was judged using tetrazolium bromide reduction assay. Cell migration was evaluated by Transwell assay. *In vitro* mineral nodule formation was assayed by von Kossa staining. Cell differentiation was examined by alkaline phosphatase activity with substrate assay. The production of osteoprotegerin was evaluated using enzyme-linked immunosorbent assay.
Results: Arecoline demonstrated cytotoxicity to cementoblasts in a dose-dependent and time-dependent manner ($P < 0.05$). Arecoline attenuated cell migration in a dose-dependent manner ($P < 0.05$). Arecoline treatment markedly suppressed cementoblast-mediated biomineralization *in vitro* compared to untreated cells at Day 8. Arecoline was found to inhibit alkaline phosphatase activity in a time-dependent manner ($P < 0.05$). In addition, arecoline decreased the secretion of osteoprotegerin in a dose-dependent manner ($P < 0.05$).
Conclusion: Taken together, these results suggest that arecoline could inhibit cell growth, migration, and differentiation in cementoblasts. Areca quid chewers might be more susceptible to the destruction of periodontium and less responsive to regenerative procedure during periodontal therapy.

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Introduction

Periodontitis is characterized by persistent gingival inflammation, breakdown of the attachment apparatus surrounding teeth, and destruction of alveolar bone. The etiology of periodontal disease is multifactorial and may be associated with life style, environmental factors, and oral habits. The habit of areca quid chewing is widespread in Taiwan, Southwest Asia, and India.[1] Although the composition of the areca quid shows regional variations, it generally consists of areca nut, betel leaf, lime, and other additives. Areca quid chewing may be one of the risk factors of periodontal diseases with poor periodontal status and predominant periodontal pathogens.[2–5] However, how areca quid chewing affects the periodontal tissue responses is not fully understood.

Areca nut is the endosperm of the fruit of *Areca catechu* tree and acts as a main component of the areca quid. Arecoline, the major alkaloid of areca nut, can cause many parasympathomimetic effects including addiction, euphoria, excessive salivation, and tremor. Arecoline has been shown to alter some adverse cellular functions on the cells derived from periodontium. Previously, studies have shown that arecoline is cytotoxic to human gingival fibroblasts[6–8] and periodontal ligament fibroblasts[9] through inhibiting cell growth, attachment, and proliferation. Arecoline was also found to downregulate expression of collagens[10] and stabilize extracellular matrix proteins[11] in human gingival fibroblasts. In addition, arecoline could increase many inflammatory and immune signals by cDNA microarray in human gingival fibroblasts.[12] Despite the above evidence, the mechanisms of arecoline-induced inhibitory effects remain to be elucidated.

Cementum is a mineralized tissue, similar in composition and properties to bone, that is synthesized by cementoblasts during tooth root formation. Cementoblasts are responsible for tooth root-cementum formation during periodontal development and regeneration. No study has evaluated the effects of arecoline on cementoblasts. In the present study, the effects of arecoline on murine immortalized cementoblast cell line (OCCM.30) were determined through measuring cytotoxicity, cell migration, mineralization, and the activities of differentiation-related proteins alkaline phosphatase (ALP) and osteoprotegerin (OPG).

Materials and methods

Cell culture

An immortalized murine cementoblast cell line (OCCM.30) was a generous gift provided by Dr. Somerman's Laboratory (University of Washington, Seattle, WA, USA). OCCM-30 cells were established by the isolation of tooth root-surface cells from transgenic mice containing a SV40 large T-antigen under the control of the osteocalcin promoter.[13] Cells were grown in Dulbecco's Modified Eagle's Medium (Gibco BRL, Gaithersburg, MD, USA), supplemented with 10% fetal calf serum and 100 U/mL penicillin G and 100 μg/mL streptomycin (Gibco BRL). Cultures were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Confluent cell layers were treated with 0.25% trypsin and 0.05% EDTA for 5 minutes.

Cytotoxicity assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Sigma Chemical Co., St. Louis, MO, USA) solution was prepared in 5 mg/mL phosphate-buffered saline just before use and filtered through a 0.22-μm filter.[14] Arecoline (Sigma Chemical Co.) was dissolved in culture medium. The cells were incubated with or without different concentrations of arecoline (20 μg/mL, 40 μg/mL, 80 μg/mL, and 160 μg/mL) for 24 hours and 48 hours, respectively. Briefly, 2×10^4 cells were seeded on a 96-well plate in 100 μL medium and left overnight to attach. Cells were treated with various concentrations of arecoline for 20 hours or 44 hours. After treatment, 50 μL MTT dye was added to each well and incubated in a CO_2 incubator for 4 hours at 37°C. To each well, 50 μL of DMSO was added. Plates were shaken until crystals were dissolved. Optical density was determined by eluting the dye with dimethyl sulfoxide and measuring absorbance at 550 nm using a spectrophotometer (Hitachi, Tokyo, Japan).

Cell migration assay

In vitro migration was tested by the Boyden chamber without Matrigel.[15] Treated cells were seeded into the Boyden chamber (Neuro Probe, Cabin John, MD, USA) on the membrane filters in the upper part at a density of 1.5×10^4 cells/well in 50 mL serum-free medium and incubated for 24 hours at 37°C. The bottom chamber contained standard medium with 20% fetal calf serum. Following incubation, the filters were air-dried for 5 hours in a laminar flow hood. The migrated cells were fixed with methanol and stained with crystal violet. Cell numbers were counted using a light microscope.

Mineralization assay

Von Kossa staining of the mineralization nodules was used to demonstrate osteogenic differentiation. Cells were seeded in six-well plates in DMEM containing 10% fetal bovine serum for confluence. Cells were cultured in mineralizing medium (DMEM containing 5% fetal bovine serum, 50 μg/mL ascorbic acid, and 10mM β-glycerophosphate) with or without