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Areca nut extracts increased the expression of cyclooxygenase-2, prostaglandin E_2 and interleukin-1α in human immune cells via oxidative stress

Lien-Yu Chang [a,b,c,e], *Hsiao-Ching Wan* [a], *Yu-Lin Lai* [b,e], *I-Chiang Chou* [b,d,e], *Yen-Ting Chen* [b], *Shan-Ling Hung* [a,b,c,e,*]

[a] *Institute of Oral Biology, National Yang-Ming University, Taipei, Taiwan*
[b] *Department of Dentistry, National Yang-Ming University, Taipei, Taiwan*
[c] *Department of Education and Research, Taipei City Hospital, Taipei, Taiwan*
[d] *Department of Stomatology, Taipei City Hospital, Taipei, Taiwan*
[e] *Department of Stomatology, Taipei Veterans General Hospital, Taipei, Taiwan*

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ABSTRACT

Background and objectives: Areca nut has been identified as a carcinogen. Inflammation reveals a strong link with tumourigenesis. The aim of this study was to investigate the effects of areca nut on the expression of the key pro-inflammatory mediators involved in malignancy, cyclooxygenase-2 (COX-2), prostaglandin E_2 (PGE_2), interleukin (IL)-1α and nuclear factor-κB (NF-κB), by human immune cells. The role of oxidative stress was also examined.

Materials and methods: Human peripheral blood mononuclear cells (PBMCs) were treated with extracts of ripe areca nut (rANE) or tender areca nut (tANE). Expression of pro-inflammatory mediators was assayed using Western blotting, reverse transcription-polymerase chain reaction, competitive enzyme immunoassay or enzyme-linked immunosorbent assay (ELISA). Activity of NF-κB was evaluated using an ELISA-based method.

Results: Both rANE and tANE enhanced the expression of COX-2, PGE_2 and IL-1α by PBMCs. The secretion of PGE_2 was induced by rANE ($\leq$20–40 μg ml^{-1}) and tANE ($\leq$160 μg ml^{-1}) significantly in a dose- and time-dependent manner. However, the above enhancing effects of ANEs could be attenuated by antioxidants. ANEs also increased the nuclear expression of the redox-sensitive factor NF-κB.

Conclusions: The results demonstrate that ANEs induced the expression of pro-inflammatory mediators mainly through the induction of oxidative stress and implicate the possibility of using antioxidants for disease prevention.

* *Corresponding author at*: Institute of Oral Biology, National Yang-Ming University, No. 155, Sec. 2, Li-Nong St., Pei-Tou, Taipei 11221, Taiwan. Tel.: +886 2 2826 7224; fax: +886 2 2826 4053.
E-mail addresses: slhung@ym.edu.tw, slhung7224@gmail.com (S.-L. Hung).

Abbreviations: ANE, areca nut extract; AQ, areca quid; PBMC, peripheral blood mononuclear cells; RT-PCR, reverse transcription-polymerase chain reaction; ELISA, enzyme-linked immunosorbent assay; NF-κB, nuclear factor-κB; COX-2, cyclooxygenase-2; PGE_2, prostaglandin E_2; IL-1α, interleukin-1α; TNF-α, tumor necrosis factor-α; IL-1β, interleukin-1β; OSCC, oral squamous cell carcinoma; NSAIDs, nonsteroidal anti-inflammatory drugs; ROS, reactive oxygen species; PDTC, pyrrolidine dithiocarbamate; AP-1, activator protein-1.

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1. Introduction

Chewing of areca quid (AQ) is an ancient and popular oral habit in countries of South and Southeast Asia and the Asia Pacific region.[1] On average, 18 pieces of quid are consumed daily by Taiwanese chewers,[2] which might cause sustained inflammatory tissue damage and promote pathologic change. Indeed, this widespread oral habit is strongly associated with oral leucoplakia, oral submucous fibrosis (OSF) and oral squamous cell carcinoma (OSCC).[3,4] Areca nut is the crucial part of AQ. In 2004, AQ chewing and areca nut were classified as group I carcinogens.[5]

Oral and oropharyngeal carcinomas comprise about half of all malignancies in India and some Asian countries.[6] In Taiwan, OSCC has been the fifth leading cause of cancer death for males.[7] Approximately 77.4–82.7% of hospital patients with OSCC are AQ chewers.[8,9] The prevalence of precancerous lesions, oral leucoplakia and OSF in AQ chewers is 7.44% and 1.58%, respectively.[10] Increased expression of pro-inflammatory molecules, such as tumour necrosis factor (TNF)-α, interleukin (IL)-1α, IL-6 and IL-8 in oral cancer patients,[11,12] and IL-1α, IL-1β, IL-6 and basic-fibroblast growth factor in the OSF tissues,[13] has been reported. Carcinogenesis, a sequential accumulation of genetic alterations by malignant cells, is also affected by stromal inflammation in the tumour microenvironment.[14] About 25% of all cancers are related to chronic unresolved inflammation.[15] Effective therapeutic manipulation of the stromal microenvironment by inhibition of the production of pro-inflammatory mediators has been demonstrated.[14,16] The finding that therapy by aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) decreases the risk of colorectal cancer has increased interest in the possibility of using anti-inflammatory agents for cancer prevention.[16]

NSAIDs function by inhibiting the cyclooxygenases (COXs), which are responsible for catalysing the synthesis of prostaglandin E_2 (PGE_2) from fatty acids.[16] COX-2, an enzyme regulated by NF-κB, could be induced by various inflammatory stimuli in human tumours.[16,17] The up-regulation of COX-2 and PGE_2 is related to cell proliferation, angiogenesis and metastasis in human malignancies.[16–18] Moreover, COX-2-derived PGE_2 is important in tumours that evade immune surveillance by re-educating infiltrating inflammatory/immune cells during tumourigenesis.[19] IL-1, the highly inflammatory molecule, together with TNF-α, can stimulate the expression of COX-2, PGE_2 and various cytokines including themselves resulting in an amplification loop of inflammation.[20,21] Prolonged expression of IL-1, the master cytokine, causes pathologic tissue destruction, angiogenesis and tumourigenesis.[20] IL-1α, one form of IL-1 that is mainly active in cell-associated patterns, is secreted in many types of tumour cells, but not in normal cells.[21] IL-1α has been demonstrated in inducing malignant cell transformation.[21] Overexpression of COX-2-derived PGE_2 and IL-1α has been demonstrated in various cancers including head and neck SCC.[11,18,22,23] Inhibition of the activity of COX-2, PGE_2 and IL-1 would be beneficial in clinical medicine for reducing the severity of chronic inflammation and cancer.[16,23,24]

Areca nut comes from the tropical palm tree, *Areca catechu*.[1] The nut used in Taiwan is fresh and tender, which is different from that used in other countries such as India where the nut used is the ripe and fully-grown one without the husk. The extract of ripe areca nut (rANE) is more cytotoxic than the extract of tender areca nut (tANE) to cultured cells.[25,26] The cytotoxicity is positively related to the levels of oxidative DNA damage.[27] The research planned to investigate the effects of both types of ANEs on immune cell function in vitro first before exploring the whole immune modulation of cancer stroma by areca chewing. Peripheral blood mononuclear cells (PBMCs), including monocytes and lymphocytes, are the main source of chronic inflammatory cells and immune effectors.[28] Previous studies show that ANEs enhance the expression of potent pro-inflammatory cytokines, TNF-α, IL-1β, IL-6 and IL-8, by human PBMCs.[25,26] This study further examined whether ANE affects the expression of COX-2, PGE_2 and IL-1α, which are key inflammatory mediators in the development of malignancy, by human PBMCs. The role of reactive oxygen species (ROS) and redox-sensitive factor NF-κB was also determined.

2. Material and methods

2.1. *Preparation of ANE*

The rANE was extracted from dried ripe areca nuts without the husk and the tANE was extracted from fresh tender areca nuts with the husk as previously reported.[27] The tANE was dissolved in calcium- and magnesium-free Hank's balanced salt solution (HBSS) (Gibco BRL Laboratories, Grand Island, NY, USA). The rANE was dissolved in 50% of dimethyl sulphoxide (DMSO) (Sigma Chemical Co., St. Louis, MO, USA) in HBSS. The final concentration of DMSO in each rANE-treated sample was <0.4%.

2.2. *Preparation of PBMCs and culture conditions*

This study was approved by the Institutional Review Board of National Yang-Ming University, Taipei, Taiwan. All volunteers signed informed consent for research use of their blood cells. PBMCs were freshly prepared from the venous peripheral blood of healthy adult volunteers (10 men and nine women; mean age 24.8 ± 2.3 years; age range: 21–31 years) without smoking using dextran sedimentation followed by Ficoll (Ficoll-paque PLUS, Amersham Pharmacia Biotech, UK) density-gradient centrifugation as described previously.[25,26] The purified PBMCs were resuspended in Roswell Park Memorial Institute (RPMI)-1640 media (Gibco BRL Laboratories, Grand Island, NY, USA) supplemented with 10% heat-inactivated foetal bovine serum, 100 U ml^{-1} penicillin G sodium, 100 μg ml^{-1} streptomycin sulphate and 0.25 μg ml^{-1} amphotericin B (Gibco BRL Laboratories, Grand Island, NY, USA). The cells were treated with different concentrations of either rANE or tANE at 37 °C for 4 or 24 h. Cells treated with DMSO (up to 0.4%) under similar conditions served as the vehicle controls. For experiments of antioxidants, PBMCs were preincubated with various concentrations of pyrrolidine dithiocarbamate (PDTC) or curcumin (Sigma Chemical Co., St. Louis, MO, USA) for 1 h followed by incubation with media only, 20 μg ml^{-1} of rANE or 80 μg ml^{-1} of tANE for 24 h. Each antioxidant was present throughout the incubation period. At the end of