Areca nut extract and arecoline induced the cell cycle arrest but not apoptosis of cultured oral KB epithelial cells：association of glutathione, reactive oxygen species and mitochondrial membrane potential.

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Abstract

There are 600 million betel quid (BQ) chewers in the world. BQ chewing is a major etiologic factor of oral cancer. Areca nut (AN) and arecoline may inhibit the growth of oral mucosal fibroblasts (OMF) and keratinocytes. In this study, AN extract (100–800 μg/ml) and arecoline (20–120 μM) inhibited the growth of oral KB cells by 36–90 and 15–75%, respectively. Exposure to arecoline (>0.2 mM) for 24 h induced G2/M cell cycle arrest of OMF and KB cells. Areca nut extract (>400 μg/ml) also induced G2/M arrest of KB cells, being preceded by S-phase arrest at 7-h of exposure. No evident sub-G0/G1 peak was noted. Marked retraction and intracellular vacuoles formation of OMF and KB cells were observed. Glutathione (GSH) level, mitochondrial membrane potential (ßm) and H2O2 production of KB cells were measured by flow cytometry. GSH level [indicated by 5-chloromethyl-fluorescein (CMF) fluorescence] was depleted by 24-h exposure of KB cells to arecoline (0.4–1.2 mM) and AN extract (800–1200 μg/ml), with increasing the percentage of cells in low CMF fluorescence. By contrast, arecoline (0.1–1.2 mM) and AN extract (800–1200 μg/ml) induced decreasing and increasing H2O2 production (by 2',7'-dichloro- fluorescein fluorescence), respectively. Hyperpolarization of ßm (increasing of rhodamine uptake) was noted by 24-h exposure of KB cells to arecoline (0.4–1.2 mM) and AN extract (800–1200 μg/ml). AN extract (100– 1200 μg/ml) and arecoline (0.1–1.2 mM) induced little DNA fragmentation on KB cells within 24 h. These results indicate that AN ingredients are crucial in the pathogenesis of oral submucous fibrosis (OSF) and oral cancer by differentially inducing the dysregulation of cell cycle control, ßm, GSH level and intracellular H2O2 production, these events being not coupled with cellular apoptosis.