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Sanguinarine, a Benzophenanthridine Alkaloid, Induces Apoptosis in HCT-116 Human Colon Cancer Cells through a Reactive Oxygen Species-Mediated Mitochondrial Pathway

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Sanguinarine is a benzophenanthridine alkaloid that is derived from the root of Sanguinaria canadensis and other poppy fumaria species, and is known to have antimicrobial, antiinflammatory and antioxidant properties.In the present study, we investigated the biochemical mechanisms of apoptosis induction by sanguinarine in human colon cancer cells. Growth inhibition of human colon cancer HCT-116 cells was assessed by MTT assays. The determination of apoptotic cell death was performed by flow cytometry analysis, agarose gel electrophoresis and DAPI staining. The effects of sanguinarine on the expression of apoptotic-regulated gene markers were detected by Western blot analysis and colorimetric assay kits. At a 0.6-1.2 uM dose-level, sanguinarine significantly enhanced apoptosis. Sanguinarine caused apoptosis in HCT-116 cells through the down-regulation of MMP, Bcl-2, cIAP-1, xIAP, tBid. Sanguinarine also activated caspases-3, -8, -9. Moreover, the quenching of ROS generation by NAC, a scavenger of ROS, reversed the sanguinarine-induced apoptosis effects via inhibition of ROS production, MMP, Bcl-2, cIAP-1, xIAP, tBid expression and the subsequent activation of caspases. These observations clearly indicate that ROS are involved in the early molecular events in the sanguinarine-induced apoptotic pathway. Our data imply that sanguinarine-induced ROS are key mediators of MMP, which leads to the downregulation of MMP by caspase activation, culminating in apoptosis. [This work was supported by a grant from the Personalized Tumor Engineering Research Center (PT-ERC) and the Korea Science and Engineering Foundation (KOSEF).]

Key words: Apoptosis, Sangunarine, ROS, MMP

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The Combination Therapy with Genistein and Hydroxypheophorbide-**α**-Mediated Photodynamic Therapy Induced Apoptosis in CaSki Cervical Cancer Cells

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Photodynamic therapy (PDT) is a treatment for cancer involving three key components (a sensitizing compound, light, and tissue oxygen). In this study we applied photo-treatment to cancer cells with 2 J/cm^2 of red light after sensitizing with 9-hydroxypheophorbide-α (9-HpbD-α), a new chlorophyll-derived photosensitizer. We have investigated the cytotoxic and apoptotic effects of 9-HpbD-α-induced PDT in cervical cancer cells, the enhancing effect of genistein in PDT, and explored the molecular mechanisms of E6 or E7 oncogenes, apoptotic signaling molecules, and ER stress. Co-treatment downregulated the transcripts of the E6*I, E6*II, and E7 oncogenes. Combined treatment with PDT and genistein showed typical apoptotic features, i.e., apoptotic bodies. To elucidate the mechanism of combination treatment-induced apoptosis, various mediators of apoptosis were investigated. Activation of caspase-8, caspase-3, and PARP weredistinct after combination treatment. Furthermore, ER stress-related proteins, such as CHOP and GRP78, were activated after combination treatment. We conclude that genistein sensitizes CaSki cells to apoptosis treated with PDT by 9-HpbD-α (0.59 ug/ml) through mechanisms that involve downregulation of the E6*I, E6*II, and E7 oncogenes, activation of caspase-8/capase-3, and ER stress.

Key words: Genistein, CaSki cervical cancer cells, apoptosis, photodynamic therapy , 9-hydroxypheophorbide-α (9-HpbD-α)