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Effects of Andrographolide and Taxifolin on Cell Proliferation, Cell Cycle Progression and Apoptosis of Prostate Carcinoma DU145 Cells

穿心蓮內脂和花旗松素對前列腺癌細胞DU145增殖，週期和凋亡的影響

Submitted to
Department of Biology and Chemistry
生物及化學系
in Partial Fulfillment of the Requirements
for the Degree of Master of Philosophy
哲學碩士學位

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February 2011
二零一一年二月

ABSTRACT

Natural products have been playing a domain role in cancer chemotherapy and prevention in the past 30 years. Traditional Chinese medicine (TCM) with thousands years of history is a huge source for discovery and investigation of effective natural herbal products. A lot of compounds isolated or derived from TCM have been suggested to possess the property of inducing cell cycle arrest, cell differentiation and apoptosis in various kinds of cancer cells.

Andrographolide (Andro) which is the main bioactive component from *Andrographis paniculata* (穿心蓮) has been reported to have many biological effects including anti-proliferative effect on several tumor cell lines. However, there is no detailed study about the biological effects of Andro on androgen refractory prostate cancer cells. Taxifolin (Taxi), a dihydroflavonol belongs to flavonoids group, together with its glycosides are commonly found in many species of medical herbs. In recent years, experimental studies have provided growing evidences for the protective effect of flavonoids against cancer because of their beneficial actions on multiple cancer-related biological pathways (e.g. carcinogen bioactivation, cell-signaling, cell cycle regulation, angiogenesis, oxidative stress, inflammation). Although the reports on flavonoids and cancer are still limited and conflicting, some protective associations have suggested flavonoid-rich food for cancer protection.

Results in the present study showed that Andro inhibited cell proliferation of androgen independent prostate cancer cell line DU145 in a time and dose-dependent manner, with the IC_{50} value (48 h) of 13.70 μM. On the other hand, Andro exhibited little growth inhibitory effect on noncancerous human fibroblast

Hs27 (IC_{50}>500 μM, 48 h). Cell cycle analysis demonstrated that, at low concentration (<= 40 μM), Andro-treated DU145 cells accumulated at G2/M phase dose-dependently. Immunoblot of Phospho-Histone H3 (Ser10) antibody (mitotic marker) further revealed that the G2/M accumulation of DU145 cell was caused by cell cycle arrest at mitotic phase. Additionally, microtubule network was visualized by immunostaining of tubulin, which suggested that Andro treatment leaded to the formation of abnormal spindle-chromosome structure resulting in cell arrest at prometaphase. However, *in vitro* microtubule assembly assay indicated that Andro did not interact directly with microtubule. Double staining of AnnexinV-FITC / PI showed that Andro also induced apoptosis dose-dependently, with the highest apoptotic rate after 48-hour treatment. High concentration (80 μM) of Andro treatment directly induced cell death without a marked alteration of cell cycle distribution within the first 24 hour. Western blotting analysis revealed that Andro exposure triggered several cell cycle regulation pathways, including up-regulation of cyclin B1 and cyclin-dependent kinase (CDK) inhibitor p21(Waf1/Cip1), dephosphorylation on Tyr15 of Cdc2 and phosphorylation of Wee1, Myt1 and Cdc25C, which involved in the process of cyclin B/Cdc2 complex activation and led to cell accumulation in mitosis. Andro-induced apoptosis was associated with activation and cleavage of poly (ADP-ribose) polymerase (PARP), caspase-7, caspase-9 and caspase-3 related to mitochondria apoptotic pathway.

Taxi exhibited low anti-proliferative effect on DU145 cell line (IC_{50}>500 μM, 48 h). On the other hand, combination of 100 μM Taxi with Andro significantly enhanced the growth inhibitory effect of the latter on DU145 cells. It was found that combinated treatments of 100 μM Taxi and Andro (10–40 μM) markedly increased

G2/M accumulation in DU145 cells compared to treatments with Andro-alone, through rising of mitotic index (approximately twice with 20 μM Andro treatment). Quantification of apoptosis with flow cytometry revealed that Andro-induced apoptosis and cell death was also promoted potently by synchronous treatment of Taxi, whereas exposure to Taxi alone did not exhibit marked alteration on cell morphology, cell cycle distribution or cell viability. Western blotting analysis revealed that the mitotic rate increased by combination treatment was related to up-regulation of cyclin B/Cdc2 mitotic complex and CDK inhibitor p21; the enhanced apoptosis was associated with increases of PRAP, caspase-7, and caspase-9 activation. The increase of twisted and elongated mitotic spindle after the combined treatment suggested that anti-microtubule activity of the two combined compounds was involved as a remarkable enhanced microtubule polymerization was observed.

This study investigated the biological effect of Andro and Taxi on cell morphology proliferation, cell cycle, apoptosis in androgen-independent prostate cancer cell DU145, and the synergetic/additive anticancer effect with combination treatment. Hence, Andro might be effective on prostate cancer treatment and low cytotoxic Taxi might be a promising additive in combined drug treatment of prostate cancer. This study also provided experimental evidences for the potential treatment of cancer by dietary-flavonoid in combination with anti-cancer compounds.

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