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RUNGKAN POOTRAKRONCHAI : APOPTOSIS INDUCTION BY VR-3848 ISOLATED FROM *EUPHOBIACEAE* IN A HUMAN LUNG CANCER CELL LINE. THESIS ADVISOR: KULAWEE SUJARIT, PH.D. PAWINEE PIYACHATURAWAT, PH.D., VICHAI REUTRAKUL, PH.D., SONGSAK PETMITR, PH.D. 122 p. ISBN 974-664-014-3.

It has been demonstrated that a variety of anticancer drugs inhibit the growth of carcinoma cells by induction of apoptosis. VR-3848 is a potent-cytotoxic-unknown compound purified from *Euphobiaceae,* a tropical Thai plant. The concentration of VR-3848 that corresponds to half of the viability (GI_{50}) was 10 nM. The ability of VR-3848 to initiate apoptosis was investigated in a human lung (LU-1) cancer cell line. Treatment of cells with VR-3848, GI_{50} (10 nM), 10x GI_{50} (100 nM), and 20x GI_{50} (200 nM) or vinblastine (a positive control), 2xGI_{50} (200 nM) between 3 and 48 hours caused morphological changes consistent with the induction of apoptosis. Apoptosis induced by VR-3848 detected by 4′, 6-Diamino-2-phenylindole (DAPI) staining and visualized by a fluorescence microscope was time- and –dose dependent. The peaks of apoptosis were seen at 48 hours incubation period up to 30% and 40% in cells treated with VR-3848, 100 nM and 200 nM, respectively. In addition, studies by agarose gel electrophoresis, a characteristic ladder of DNA fragments in multiples of 180-200 base pairs, was observed in DNA extracted from cells treated with VR-3848, 200 nM for 48 hours. The results were similar in cells treated with vinblastine 200 nM for 48 hours. To examine whether the cysteine protease, CPP32 (caspase-3), contributes to the VR-3848-induced apoptosis, the expression of mRNA for CPP32 in LU-1 cells was performed by RT-PCR. The results revealed that both control and treated LU-1 cells expressed the almost same levels of mRNA for CPP32. Importantly, at the apoptosis-inducing concentration, VR-3848 also induced the activation of caspase-3 between 3 and 48 hours. Furthermore, 5 μM of a specific inhibitor of caspase-3-like protease, Ac-DEVD-CHO, significantly blocked CPP32 activity. In summary, the findings demonstrate that VR-3848 may induce cell death in LU-1 cell lines via apoptosis which is mediated by the activation of caspase-3.