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PRE-CLINICAL EVALUATION OF CINOBUFOTALIN AS A POTENTIAL ANTI-OVARIAN CANCER AGENT

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Abstract: Cinobufotalin (CINO), a cardiotonic steroid (CTS) or bufadienolide, is extracted from the skin secretions of the traditional Chinese medicine giant toads (Chan su). CINO has been used as a cardiotonic, diuretic and a hemostatic agent. Previously we have shown that CINO inhibits the cytotrophoblast cell function. Recently other study has shown that CINO inhibits A549, a lung cancer cell function. In this study, we assessed the effect of CINO on three different ovarian cancer cell lines; SK-OV-3, CRL-1978 and CRL-11731 and tumor xenograft model in nude mice. We evaluated the effect of CINO on three ovarian cancer cells SK-OV-3, CRL-1978, and CRL-11731 function in vitro. Each Cell lines were treated with different concentrations of CINO (0.1, 1, 5 and 10 µM). For each cell line cell proliferation, migration and invasion were measured by using a CellTiter Assay (Promega), Cytoselect Assay (Cell Biolabs) and by using a FluoroBlock Assay (BD) respectively. Proliferating Cell Nuclear Antigen (PCNA) was also evaluated in cell lysates of CINO treated these 3 ovarian cancer cells by western blot analysis. Cell Cycle arrest and Cell viability were determined by fluorescence-activated cell sorting (FACS) analysis. We also performed Annexin V staining on CINO treated these 3 ovarian cancer cell lines by immunofluorescence to evaluate the pro-apoptotic protein expression. In addition mitochondrial membrane potential has also been measured for all these 3 ovarian cell lines after CINO treatment using MMP kit, by FACS analysis. Male nude mice will be introduced to establish xenograft tumor model of three ovarian cancer cell lines: SK-OV-3, CRL1173 and CRL1978 cells (five million cells in 0.1 mL of culture medium) were subcutaneously injected at the right thigh of nude mice, and treated when the tumors reached an average volume of 200–300 mm^3. Animals were randomized into 3 groups for each of three cell lines, total 9 groups with 10 mice each group: (a) vehicle; (b) 1.0 mg/kg of CINO; (c) 5.0 mg/kg of CINO. CINO were injected intraperitoneally (i.p.) twice daily for 1 weeks. The mice were examined daily for toxicity/mortality relevant to treatment, and the tumor was measured with a caliper once a week for up to 5 weeks. Mice body weight and mice survival (at week 5) were also be recorded. The tumor volume (in mm^3) was calculated by the formula: volume = (width) 2 X length/ 2, and the tumor growth curve were presented. Concentration of CINO at 0.5µM inhibit SK-OV-3, CRL-1978, and CRL-11731 ovarian cancer cells proliferation, migration and invasion without cell death and loss of cell viability but cell viability differs for each cell line. Each cell lines differ in response to CINO doses for PCNA expression as well as Annexin V pro-apoptotic protein expression. CINO decreases mitochondrial membrane potential for SK-OV-3 but for CRL-1978 and CRL-11731 increases in response to CINO treatment. CINO administration didn't significantly affect mice body weight, indicating the relative safety of this regimen. Thus, CINO inhibited ovarian cancer cells xenograft growth in vivo and improved mice survival. The results of this study suggest that CINO might be further investigated as a novel anti-ovarian cancer agent.