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Cinobufotalin as a Novel Agent to Inhibit *in Vitro* Epithelial Ovarian Cancer Cell Proliferation, Migration and Invasion

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Abstract

Objective: Cinobufotalin (CINO), a cardiotonic steroid (CTS) or bufadienolide, is extracted from the skin secretions of giant toads and is utilized in traditional Chinese medicine (Chan Su). CINO has been used as a cardiotonic, diuretic and a hemostatic agent. Recently, CINO has been shown to inhibit lung cancer cell function and has been implicated in several other disease processes. In this study, we pursued the potential anticancer application of CINO using the ovarian tumor cell line SK-OV-3. Study Design: We evaluated the effect of CINO on cultures of SK-OV-3. Cells were treated with 0.1, 0.5, 1, 5, and 10 μM CINO. Cell proliferation, migration, invasion, and viability were measured using commercially available kits. Cell cycle progression was evaluated by a Cell Cycle Phase Determination Kit. Apoptosis was evaluated by an Apoptotic Blebs Assay Kit; cell cycle arrest and apoptotic signaling were determined by fluorescence-activated cell sorting (FACS) analysis. Results: CINO at ≥ 0.5 μM inhibited SKOV-3 cell proliferation, migration, and invasion ($p < 0.05$). There was a higher ($p < 0.05$) percentage of S phase cells in groups treated with CINO at 0.5 μM. CINO at ≥ 0.5 μM down regulated expression of Proliferating Cell Nuclear Antigen (PCNA) and

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caused cell death. Conclusion: This data demonstrates that CINO impairs SK-OV-3 cell function via cell cycle arrest and apoptotic signaling, suggesting CINO be further investigated as a novel anti-ovarian cancer agent.

Keywords

Cinobufotalin, Ovarian Cancer, SK-OV-3, Apoptosis, Cell Cycle Arrest

1. Introduction

Advances in medicine have led to improved diagnosis and treatment of many gynecologic malignancies, but the overall survivability from ovarian neoplasms has seen little improvement [1]. Ovarian cancer is the 8th most common cancer; however, it is the 5th most common cause of death in women in the United States (US), with approximately 21,000 new cases and over 13,000 deaths per year [2]. Many factors influence the complexity of this disease including the relatively large number of histologic and pathologic subtypes, each with its own biologic behavior. More than 85% of malignant ovarian neoplasms are epithelial in origin and, of those, more than 50% are serous or mucinous cystadenocarcinoma [3]. Little is known about the molecular and biologic behaviors of these neoplasms, which has led to barriers in the screening, and treatment of this disease [4]. Current resources for screening are limited to Cancer Antigen 125 (CA125) and ultrasonography, which are only applicable in high-risk patient populations. These modalities are nonspecific and limited in their application. For example, CA125 is an antigen released by ovarian epithelial cancers but is also elevated in other cancers including pancreatic, breast, and bladder, as well as the benign disease-like endometriosis. Ultrasonography is a second line test that, when used in combination with CA125, has improved the specificity and positive predictive value of screening for ovarian cancer but is still not indicated for routine screening in the general patient population [5]. Carriers of the Breast Cancer (BRCA) 1 and 2 gene mutations have been identified as a target population for ovarian cancer screening. Many more genetic markers for increased ovarian cancer risk are being investigated but remain in their infancy with respect to their clinical utility [6].

Many factors contribute to the prognosis of malignant epithelial neoplasms including the stage and histologic grade. The earliest signs and symptoms are often vague gastrointestinal or genitourinary complaints resulting from advanced disease and compression of surrounding structures. As a result, most cases go undiagnosed until later stages. Early detection is often incidental during workup for unrelated problems. Because of this difficulty in improving early diagnosis, much of the ongoing research has been on treatment. Current treatment guidelines for malignant epithelial neoplasms are based on staging and debulking with adjuvant chemotherapy serving a role in treatment of at least Stage IC through Stage III disease. Because ovarian cancer is surgically staged, the amount of residual disease after optimal debulking affects prognosis. To optimize tumor cell kill and minimize chemotherapy toxicities, platinum-based combination chemotherapies are standard of care, usually carboplatin and paclitaxel [7] [8]. There is a 70% - 80% response rate for advanced Epithelial Ovarian Cancer (EOC), but less than a 25% 5-year survival [1]. Because of poor survivability, there is much interest in optimizing first-line chemotherapy regimens and providing proper maintenance therapy. Therefore, we undertook these studies on cultured SK-OV-3 cells, to evaluate the possible role of Cinobufotalin (CINO) in improving current EOC therapies and advancing our knowledge of the pathogenesis of this disease.

Our lab is familiar with CINO and its role in the pathology of preeclampsia by influencing Cytotrophoblast (CTB) cells through various cell cycling pathways [9]. This cardiotonic steroid (CTS) has also been isolated in traditional Chinese herbal medicines used in the treatment of cancer and has been studied with *in vitro* and *in vivo* models for its effects on several disease processes [10]-[16]. Cardiotonic steroids have been utilized in medical therapies for centuries. Classically, digitalis which is derived from the foxglove plant has been the hallmark for this class of compounds in medicine. However, many cultures have been using herbal supplements for a variety of health conditions including cancer that coincidently have been found to contain CTSs. This group of compounds is known to have action on the Na^+/K^+-ATPase. CINO in particular belongs to a class of CTS called bufadienolides, which have been shown to be weak inhibitors of this enzyme with presumably fewer renal and cardiac side effects [17]. Furthermore, CINO has been found to have anti-tumor effect on a variety of cancers

across several laboratories. It demonstrates anti-lung cancer properties *in vitro*, suppresses endometrial carcinoma xenografts in a nude mouse study, and has been studied as a combination therapy with cisplatin for hepatocellular carcinoma in another nude mouse study [18]-[20]. The heterogeneous tumor suppression property this compound shows makes it an exciting prospect to study in ovarian cancer. Several hypothesis at establishing a mechanism of action for this compounds tumor suppression have been proposed, but there is still little consensus. Proposed mechanisms include caspase dependent cell death [21], DNA modulation of ribonucleotide reductase subunit M2 [18], and cyclophilin D-dependent mitochondrial permeability transition pore opening [19]. The menagerie of mutations found in these unrelated cancers likely contributes to the variety of mechanisms that have been found.

Ovarian cancer cell line SK-OV-3 (American Type Culture Collection [ATCC]® Human Tissue Bank [HTB] 77™) is the target of our study. It is a hypodiploid cell line isolated from an epithelial ovarian adenocarcinoma in a 64-year-old Caucasian woman. We utilized this cell line as a model to study the effects of CINO on cell proliferation, invasion, and migration in culture [22].

2. Experimental Design and Methods

***SK-OV*-3 *Cell Culture*:** The SK-OV-3 cell line is available from ATCC as HTB-77. This is a hypodiploid human cell line with a modal chromosome number of 43 (occurring in 63.3% of cells) and a range of 42 to 45. The rate of higher ploidies is 32%. The del(1)(q21), der(13)t(1;?;13) (q11;?;q34), der(11)t(11;?) (q12), del(10)(q22) and 3 other marker chromosomes are common to most cells, and 3 others are found only in some cells. One N11 has the HSR **(**Homogeneously Staining Regions) segment from p11 to the distal end. The normal N10, N12, N15, N17 and N19 are absent. Others are either single or paired [23]. There are from 1 to 6 rearranged and unassignable chromosomes. The X chromosome is either single or paired. The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. To make the complete growth medium, the fetal bovine serum is added at a final concentration of 10%. Cells are best incubated at 37˚C, 5% CO_2, and 99% humidity (Fisher, Isotemp CO_2 Incubator). These cells are to be managed at biosafety level 2 and represent a minimal hazard for personnel. The CINO used in culture is purchased from Enzo Life Sciences and diluted into Dimethyl sulfoxide (DMSO) for treatment of cultures. The DMSO diluent is used as a control treatment.

***Effect of CINO on SK-OV*-3 *Cell Proliferation*:** Cell proliferation was measured using a CellTiter Assay (Promega, Madison, WI) as in our previous trials with cultured cells [24] [25]. The assay kit uses a colorimetric method for determining the number of viable cells. Cells were treated with 0, 0.1, 0.5, 1, 5, and 10 nM CINO for 48 hours to better delineate in vitro efficacy. After CellTiter 96 is added to the cells, absorbance at 490 nm is measured on a plate reader (SPECTRAmax 340PC384 Microplate Spectrophotometer, Molecular Devices). Cell viability was measured using a CellTiter Assay (Promega, Madison, WI).

***Effect of CINO on SK-OV*-3 *Cell Migration*:** Cell migration was measured using a CytoSelect Assay (Cell Biolabs) with treatments of 0, 0.1, 0.5, 1, 5, and 10 nM CINO for 48 hours at the optimal oxygen concentration (5%). After the CyQuant GR Dye solution is added to the cells, fluorescence is measured at 480 nm/520 nm on a fluorescence plate reader (CytoFluor Series 4000 Fluorescence Multi-Well Plate Reader, Applied Biosystems, Foster City, CA).

***Effect of CINO on SK-OV*-3 *Cell Invasion*:** Cell invasion was measured using a FluoroBlok Assay (BD). Cells were treated with 0, 0.1, 0.5, 1, 5, and 10 nM CINO for 48 hours at the optimal oxygen concentration. After Calcein-acetoxymethyl is added to the cells, fluorescence is measured at 490 nm/700 nm on a fluorescence plate reader (CytoFluor Series 4000, Applied Biosystems, Foster City, CA).

Cell viability was measured using a CellTiter Assay ((Promega, Madison, WI)) and treatments of 0, 0.1, 0.5, 1, 5, and 10 100 nM CINO for 48 hours. After the CellTiter-Blue reagent is added to the cells, absorbance at 520 nm is measured on a plate reader (SPECTRAmax 340PC384, Molecular Devices, LLC, Sunnyvale, CA).

***Effect of CINO on proliferating cell nuclear antigen* (*PCNA*) *Expression*:** After the treatment, the media was removed from cells and a lysis buffer (Cell Signaling Technology) containing 50 mM Tris at pH 7.4, 50 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.3 mM Na-orthovanadate, 50 mM NaF, 1 mM DDT, 10 µg/ml leupeptin, and 5 µg/ml aprotinin was added to the cells. Cells were scraped and put into tubes. Protein concentrations were determined by Bicinchoninic Acid reagent (Pierce, Rockford, IL). An equal amount of protein in each sample was separated using NuPAGE Novex 4% - 12% Bis-Tris gels (Invitrogen, Carlsbad, CA) and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk and probed with PCNA (Santa Cruz), and

β-actin (Santa Cruz) antibodies. After incubation with the corresponding secondary antibody, proteins were visualized with chemiluminescence detection system (Pierce, Thermo Fisher Scientific, Carlsbad, CA). The intensity of the bands was determined using ImageQuant LAS 4000 (GE Healthcare, Life Sciences, Pittsburgh, PA). The expression of PCNA protein was quantified by densitometry analysis using Image J software where the target protein (PCNA) is normalized to a structural protein (*β*-actin) to control between groups.

***Effect of CINO on Cell Cycle and Apoptotic Signaling*:** Cell cycle progression was evaluated by a Cell Cycle Phase Determination Kit (Cayman Chemical, Ann Arbor, MI) and apoptosis was evaluated by an Apoptotic Blebs Assay Kit (Cayman Chemical). Cells were treated with 0, 0.1, 0.5, 1, 5, and 10 nM CINO for 48 hours at the optimal oxygen concentration. The cell cycle analysis and apoptotic blebs assay is performed on an Accuri C6 Flow Cytometer (Accuri Cytometers, Inc., Ann Arbor, MI). Cellular DNA content and staining intensity is analyzed with FCS Express software (De Novo Software, Los Angeles, CA). Cell cycle arrest and apoptotic signaling was determined by fluorescence-activated cell sorting (FACS) analysis.

***Statistical Method*:** Data from CINO-treated in vitro experiments were compared to those from basal DMSO-treated controls using analysis of variance (ANOVA) with repeated measures design as CINO dosages are varied within each experiment. Experiments were repeated 5 times to produce the replicates. Duncan's post hoc testing was used to detect differences between the different CINO dosages. A p-value of less than 0.05 was considered significant. Duncan's post hoc testing was used to assess dose differences.

3. Results

CINO, at *in vitro* concentrations greater than 0.5 micromole, has been shown to inhibit SKOV-3 cell proliferation, migration, and invasion ($p < 0.05$) (**Figures 1-3**). **Figure 1** represents the dose dependent decreased proliferation of SKOV-3 in cultures treated with CINO. At a dose of 0.5 µM, a statistically significant decrease in proliferation is seen as measured by absorbance at 490 nm ($p < 0.05$). No difference was observed between concentrations 5 - 10 µM allowing us to limit our therapeutic range between 0.5 - 1 µM. **Figure 2** and **Figure 3**

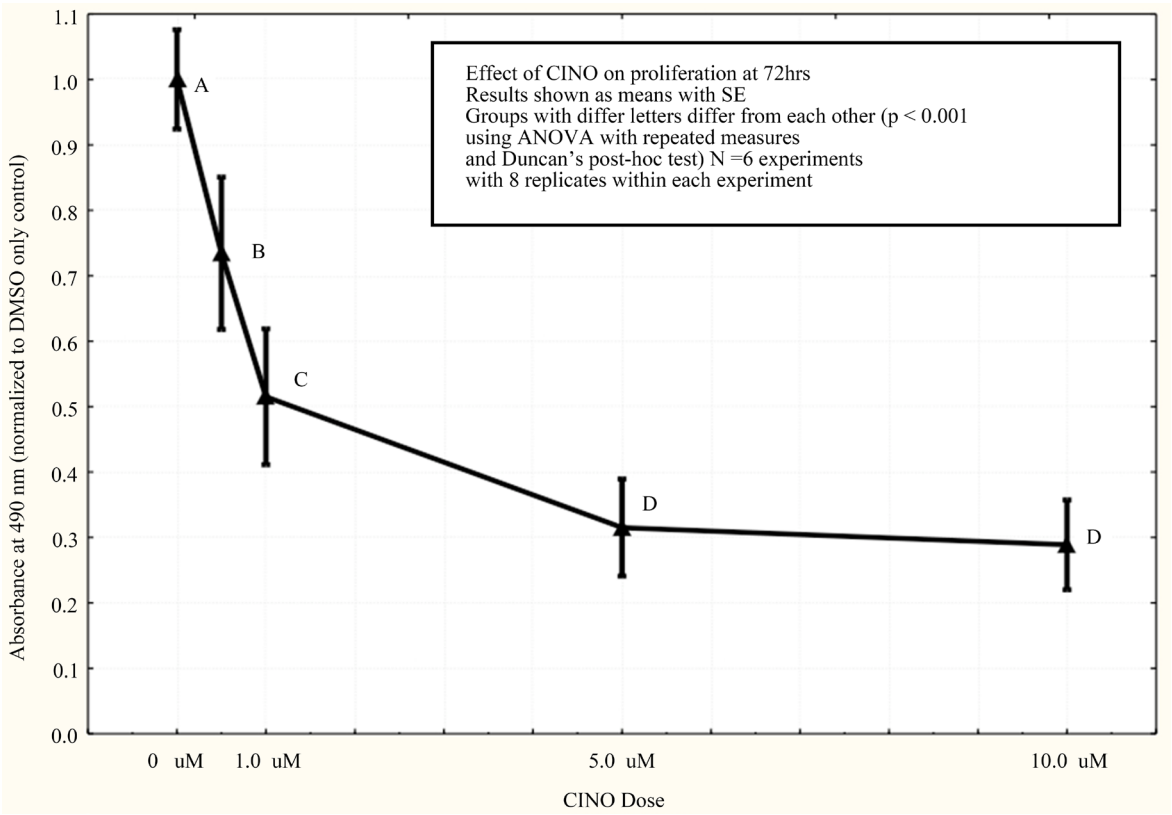


Figure 1. CINO treatment on proliferation of SK-OV-3 cells measured as absorbance at 490 and normalized to the average reading with DMSO only. CINO decreases proliferation in dose response manner.

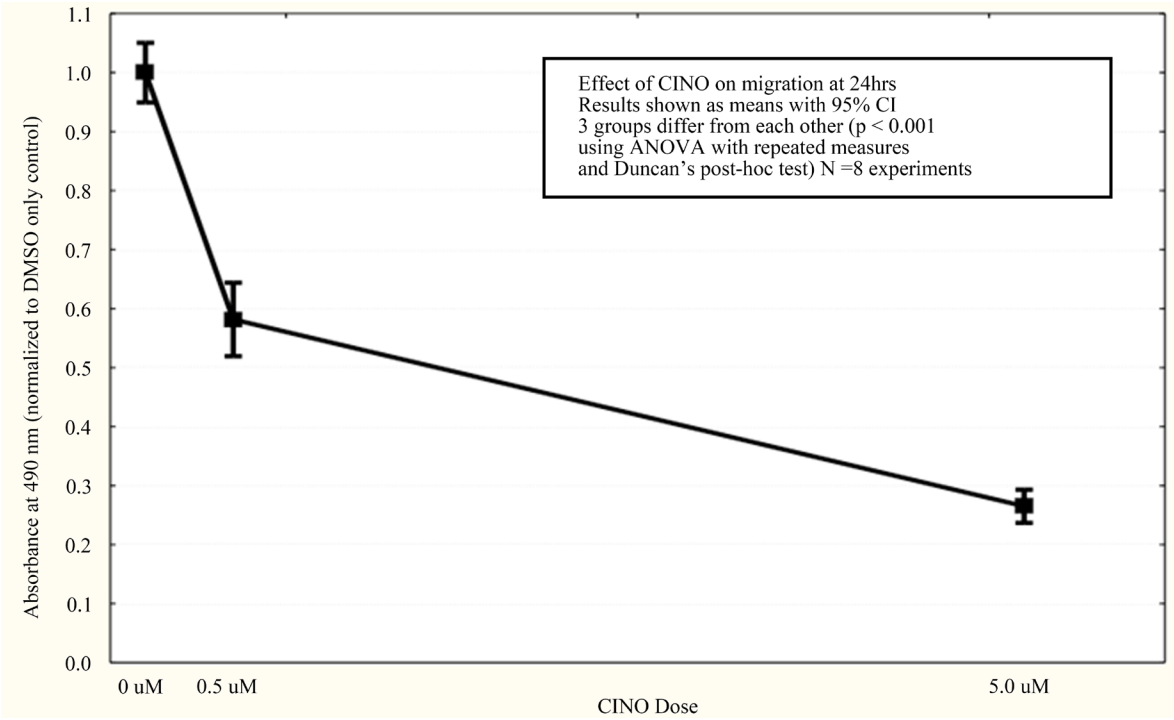


Figure 2. CINO treatment on SK-OV-3 cells migration measured as absorbance at 490 and normalized to the average reading with DMSO only. CINO decreases migration in dose response manner.

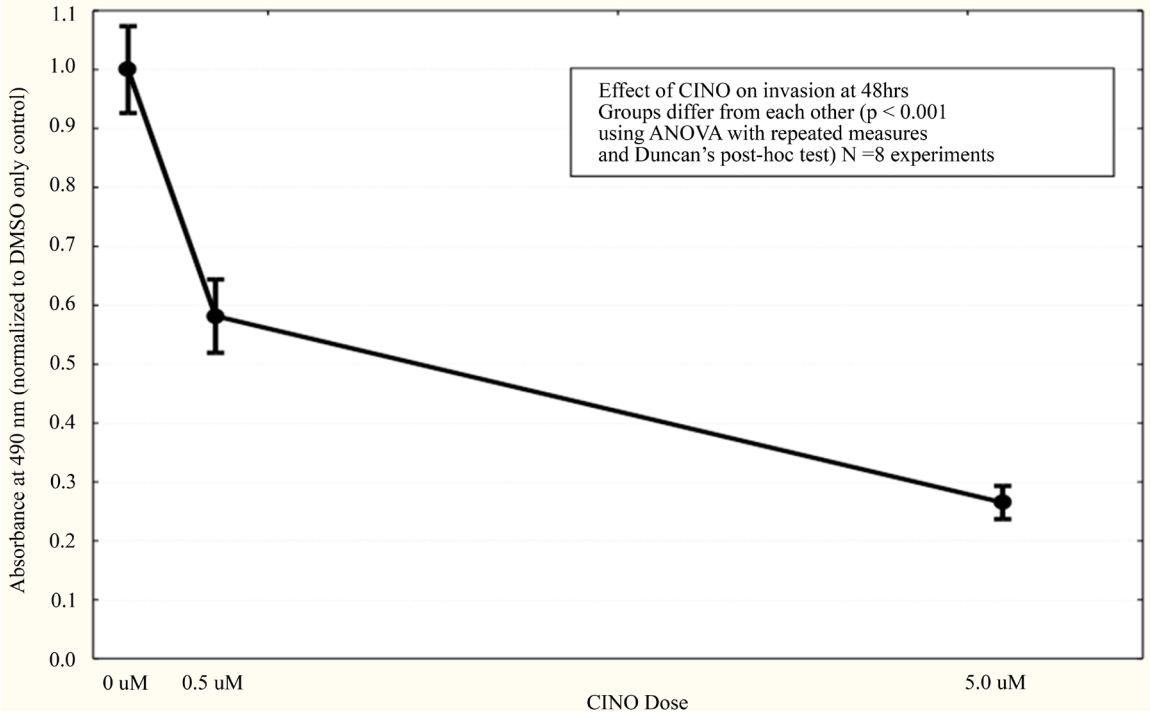
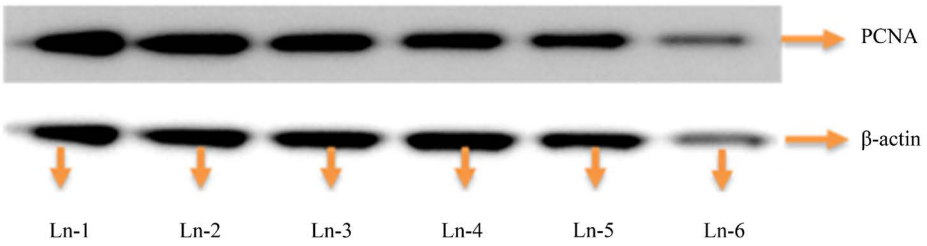


Figure 3. CINO treatment on SK-OV-3 cells invasion measured as absorbance at 490 and normalized to the average reading with DMSO only. CINO decreases invasion in dose response manner.

show a similar trends of statistically significant decrease in migration and invasion, respectively, at a dose of 0.5 μm ($p < 0.05$). The anti-proliferative capacity of CINO on the cells was not due to a cytotoxic effect of the ste-

roid, as evaluated by a cell viability assay where fewer than 3% were non-viable in all conditions (data not shown). Furthermore, it was shown to down regulate PCNA expression and cause cell death (**Figure 4-6**). **Figure 4(a)** is a Western Blot (WB) showing the dose dependent decrease in expression of PCNA normalized to Beta Actin. **Figure 4(b)** is the densitometry analysis of the WB showing the most pronounced inhibition between concentrations of 0.5 and 5 μM of CINO. **Figure 5** and **Figure 6** are the FACS data showing a similar dose dependent trend of decreased viability at a concentration of 0.5 CINO. In **Figure 5**, overlaid data from a cell viability assay show percentage of cells in S phase compared with percentage of viability. There was a higher ($p < 0.05$) percentage of S phase cells in groups treated with CINO at ≥0.5 μM. With this information, it can be assumed that CINO is eliciting its effect through the intrinsic pathway leading to apoptosis.



Ln-1 : SKOV-3 + DMSO

Ln-2 : SKOV3 + Cino (0.1μM)

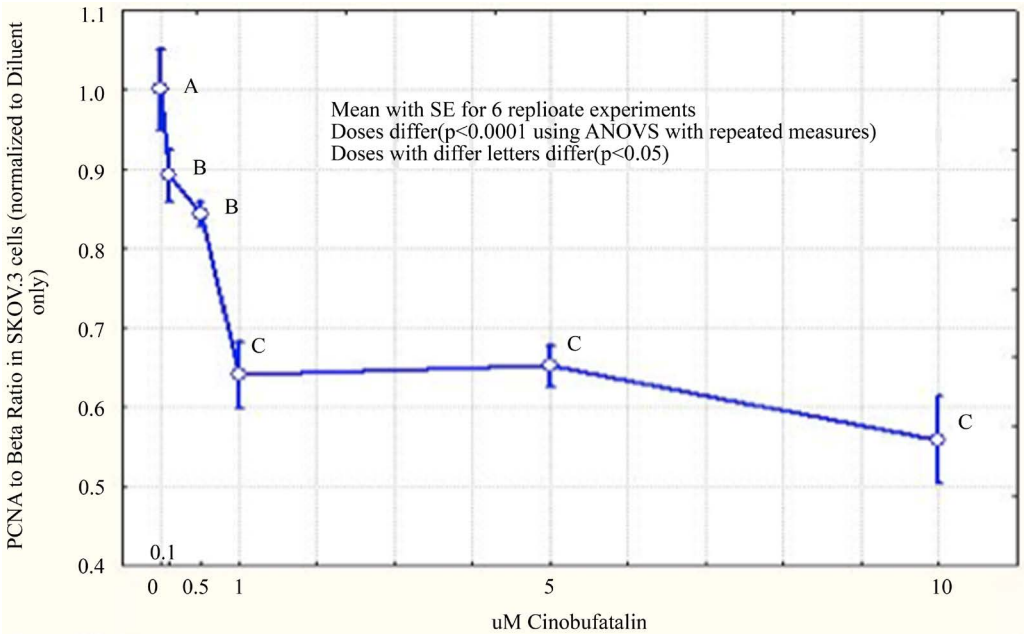
Ln-3 : SKOV3 + Cino (0.5μM)

Ln-4 : SKOV3 + Cino (1μM)

Ln-5 : SKOV3 + Cino (5μM)

Ln-6 : SKOV3 + Cino (10μM)

(a)



(b)

Figure 4. (a) WB images of PCNA; (b) densitometry of PCNA expression from WB.

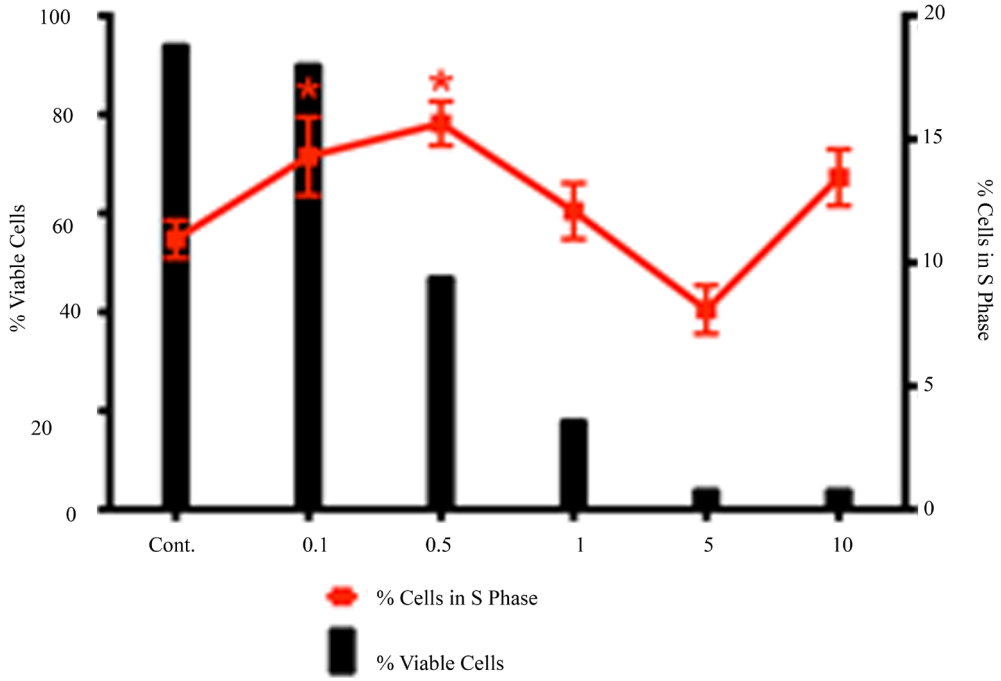


Figure 5. Cell cycle phase determination in CINO-induced SK-OV-3 cells by FACS analysis.

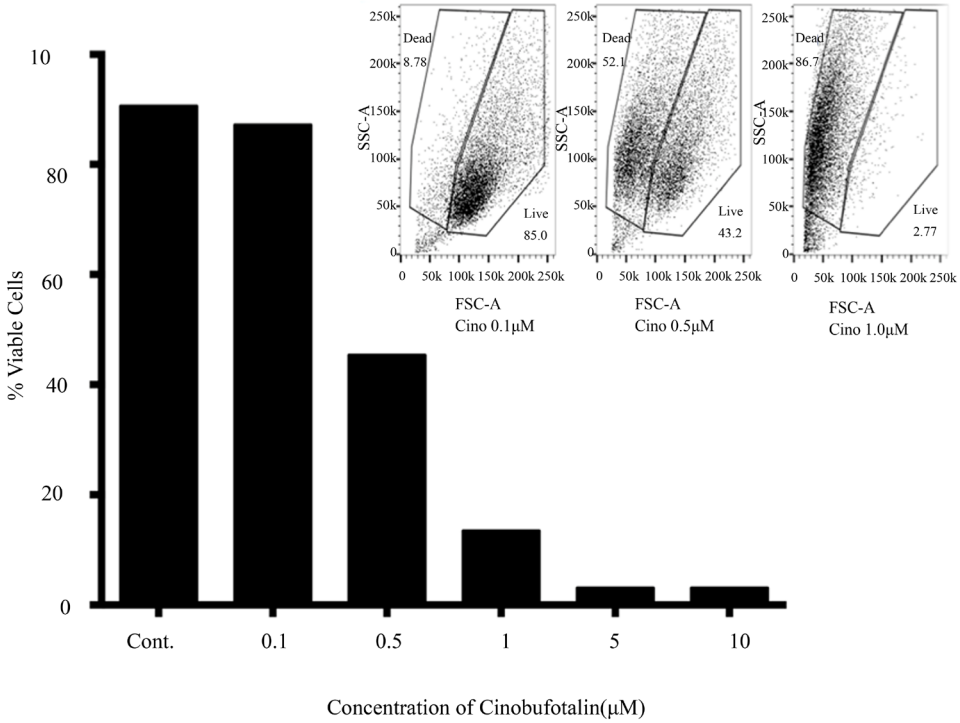


Figure 6. CINO caused the SK-OV-3 cells death evaluated by FACS analysis.

4. Discussion

In this study we have found a consistent therapeutic range between 0.5 and 5 μm. Concentrations of CINO at 0.5 inhibit proliferation, migration, and invasion without cell death and loss of cell viability. These data demonstrate that CINO in concentrations greater than 0.5 μM greatly increases apoptosis in cell lines SK-OV-3 resulting in a

loss of cell viability and decrease in PCNA expression. This is in contrast to several other studies that have been testing this compound at much higher dosages [13] [16] [19] [20], albeit in different cancers. Despite no other ovarian cancer studies utilizing CINO to compare to, a trend toward anti-tumor activity has been observed. Many advances have been made in our understanding of the role cardiotonic steroids may play in a variety of disease processes including congestive heart failure and chronic renal disease [26]. However, the realm of utilizing this class of drugs in cancer treatment, including CINO, remains relatively novel. CTS, which are produced endogenously and are present in extracts of some herbal medicines, have shown *in vivo* effects on certain tumor cell growth. We can look to history as an example that this class of drugs may have a role to play in modern medicine. Traditional herbal medicine remedies including Foxglove and Chensu have exploited the effect these medicines can have on disease processes for more than a century. We now anticipate that CINO may also play a role in the pharmacological treatment of ovarian cancer by inhibition of ovarian cancer proliferation, migration and invasion. The exact mechanism of action CINO plays at the cellular and molecular level remains to be elucidated. Like all other CTS, CINO has binding sites on Na^+/K^+-ATPase and modulates its activity. Downstream signaling cascades involving this membrane protein are complex and involve a variety of protein kinases. Furthermore, Na^+/K^+-ATPase exhibits differential sensitivity to various CTS with several tissue specific isoforms. In addition to this heterogeneous enzyme, several other cellular targets for CNIO can be inferred. We now know that CNIO suppresses cellular PCNA expression, in SKOV3, which is directly involved in DNA synthesis and repair as well as influences cellular entry into apoptosis. PCNA is a vital protein for DNA processing found in higher concentrations in S phase and serves as a cofactor for several of the polymerases involved in DNA repair and elongation [27]. With the down regulation of this protein, it can be inferred that CINO is causing rapidly dividing cancer cells undergoing unregulated DNA replication to have a diminished ability for DNA repair [26]. This can account for the inverse relationship between cell viability and percentage of cells in S phase. Our cell viability assay, which utilized apoptotic blebs as a marker for cell death, showed entry of these cells into an apoptotic pathway. However, further understanding of which apoptotic pathway is utilized and the mechanism of entry into this pathway needs continued investigation.

It is unknown if CINO is acting locally in the nucleus to directly alter DNA synthesis or if it utilizes upstream signaling pathways. The findings from this study demonstrate the complex nature of this compound. Not only can CINO directly modulate the actions of the Na/K ATPase in the classic mechanism of cardiotonic steroids, it is directly influencing the nuclear expression of proteins involved in cell cycle progression and DNA repair. We now look to gain a better understanding of the pathophysiology and mechanism of action that CINO has on EOC as well as tissue and organism specific response. In conclusion, we have shown CINO impairs SK-OV3 cell function in culture via cell cycle arrest and apoptotic signaling and suggest that CINO might be further investigated as a novel anti-ovarian cancer agent.

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Conflict of Interest

None of the authors have a potential conflict of interest in relation to this work.

References

[1] Levine, D.A. (2010) Handbook for Principles and Practice of Gynecologic Oncology. Wollters Kluwer/Lippincott Williams & Wilkins, Philadelphia.

[2] Disaia, P. (2012) Clinical Gynecologic Oncology. 8th Edition, Elsevier/Saunders, Philadelphia.

[3] Niederhuber, J.E. and Armitage, J.O. (2013) Abeloff's Clinical Oncology Review. 5th Edition, Elsevier Health Sciences, London.

[4] Thompson, M.W. and Mciness, R.R. (1991) Thompson & Thompson Genetics in Medicine. 5th Edition, W.B. Saunders, Philadelphia.

[5] Berek, J.S. and Hacker, N.F. (2004) Practical Gynecologic Oncology. 4th Edition, Wolters Kluwer Health, Philadel-

phia.

[6] Minion, L.E., Dolinsky, J.S., Chase, D.M., Dunlop, C.L., Chao, E.C. and Monk, B.J. (2015) Hereditary Predisposition to Ovarian Cancer, Looking beyond BRCA1/BRCA2. *Gynecologic Oncology*, **137**, 86-92. http://dx.doi.org/10.1016/j.ygyno.2015.01.537

[7] Schorge, J.O. (2008) Williams Gynecology. McGraw-Hill Medical, New York.

[8] Rubin, S. (2004) Chemotherapy of Gynecologic Cancers. 2nd Edition, Lippincott Williams & Wilkins, Baltimore.

[9] Pollard, T.D., Earnshaw, W.C. and Lippincott-Schwartz, J. (2008) Cell Biology. 2nd Edition, Saunders/Elsevier, Philadelphia.

[10] Puschett, J.B., Kumar, B. and Kamran Abbas, M.M. (2015) Differing Effects of Resibufagenin on Cinobufatalin-versus Marinobufagenin-Induced Preeclampsia in a Rodent Model. *American Journal of Perinatology*, **32**, 803-808.

[11] Xin, X.L., Liu, J., Ma, X.C, Wei, Q., Lv, L., Wang, C.Y., Yao, J.H. and Cui, J. (2010) Preparative Separation of Four Major Bufadienolides from the Chinese Traditional Medicine, Chansu, Using High-Speed Counter-Current Chromatography. *Natural Product Communications*, **5**, 1031-1034.

[12] Nasu, K., Nishida, M., Ueda, T., Takai, N., Bing, S., Narahara, H. and Miyakawa, I. (2005) Bufalin Induces Apoptosis and the G0/G1 Cell Cycle Arrest of Endometriotic Stromal Cells: A Promising Agent for the Treatment of Endometriosis. *Molecular Human Reproduction*, **11**, 817-823. http://dx.doi.org/10.1093/molehr/gah249

[13] Emam, H., Zhao, Q.L., Furusawa, Y., Refaat, A., Ahmed, K., Kadowaki, M. and Kondo, T. (2012) Apoptotic Cell Death by the Novel Natural Compound, Cinobufotalin. *Chemico-Biological Interactions*, **199**, 154-160. http://dx.doi.org/10.1016/j.cbi.2012.07.005

[14] Xu, K., Luo, H.Y. and Li, L.N. (2005) Clinical Study on Comprehensive Treatment of Primary Liver Cancer Mainly with Chinese Medicinal Perfusion/Embolization. *Chinese Journal of Integrated Traditional and Western Medicine*, **25**, 299-302.

[15] Xu, X.T. (1993) Clinical Study on Cinobufotalin in Treating Chronic Hepatitis B Virus Carriers. *Chinese Journal of Integrated Traditional and Western Medicine*, **13**, 473-475.

[16] Feng, K., Zhou, H.J. and Hu, Y.L. (2010) Effect of Cinobufotalin on Growth of Xenograft of Endometrial Carcinoma Cell Line Ishikawa in Nude Mouse and Its Impact on RRM2 Expression. *Chinese Journal of Integrated Traditional and Western Medicine*, **30**, 1183-1185.

[17] Bagrov, A.Y., Shapiro J.I. and Fedorova, O.V. (2009) Endogenous Cardiotonic Steroids: Physiology, Pharmacology, and Novel Therapeutic Targets. *Pharmacological Reviews*, **61**, 9-38. http://dx.doi.org/10.1124/pr.108.000711

[18] Feng, K., Zhou, H.J. and Hu, Y.L. (2010) Effect of Cinobufotalin on Growth of Xenograft of Endometrial Carcinoma Cell Line Ishikawa in Nude Mouse and Its Impact on RRM2 Expression. *Chinese Journal of Integrated Traditional and Western Medicine*, **30**, 1183-1185.

[19] Kai, S., Lu, J.H., Hui, P.P. and Zhao, H. (2014) Pre-Clinical Evaluation of Cinobufotalin as a Potential Anti-Lung Cancer Agent. *Biochemical and Biophysical Research Communications*, **452**, 768-774. http://dx.doi.org/10.1016/j.bbrc.2014.08.147

[20] Tao, C., Dan, L., Ling, F. and Peng, G. (2010) *In Vivo* and *in Vitro* Effects of QHF Combined with Chemotherapy on Hepatocellular Carcinoma. *Journal of Biomedical Research*, **24**, 161-168. http://dx.doi.org/10.1016/S1674-8301(10)60025-5

[21] Emam, H., Zhao, Q.L., Furusawal, Y., Refaat, A., Ahmed, K., Kadowaki, M. and Kondo, T. (2012) Apoptotic Cell Death by the Novel Natural Compound, Cinobufotalin. *Chemico-Biological Interactions*, **199**, 154-160. http://dx.doi.org/10.1016/j.cbi.2012.07.005

[22] Rock, J.A. and Jones, H.W. (2008) Te Linde's Operative Gynecology. 10th Edition, Wolters Kluwer Health/Lippincott Williams & Wilkins, Philadelphia.

[23] SK-OV-3. American Type Culture Collection. 2014. http://www.atcc.org/Products/All/HTB-77.aspx#documentation

[24] Uddin, M.N., Horvat, D., Glaser, S., Danchuck, S., Mitchell, B.M., Sullivan, D.E., Morris, C.A. and Puschett, J.B. (2008) Marinobufagenin Inhibits Proliferation and Migration of Cytotrophoblast and CHO Cells. *Placenta*, **29**, 266-273. http://dx.doi.org/10.1016/j.placenta.2007.12.009

[25] Uddin, M.N., Horvat, D., Glaser, S., Mitchell, B.M. and Puschett, J.B. (2008) Examination of the Cellular Mechanism by Which Marinobufagenin Inhibits Cytotrophoblast Function. *The Journal of Biological Chemistry*, **283**, 17946-17953. http://dx.doi.org/10.1074/jbc.M800958200

[26] Bagrov, A.Y., Shapiro, J.I. and Fedorova, O.V. (2009) Endogenous Cardiotonic Steroids: Physiology, Pharmacology, and Novel Therapeutic Targets. *Pharmacological Reviews*, **61**, 9-38. http://dx.doi.org/10.1124/pr.108.000711

[27] Tsurimoto, T. (1999) PCNA Binding Proteins. *Frontiers in Bioscience*, **4**, D849-D858.