Original Research Article

5, 7-Dihalo-8-quinolinol complex inhibits growth of ovarian cancer cells via the downregulation of expression of Wip1

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Abstract

Purpose: To assess the cytotoxic effect of 5, 7-dihalo-8-quinolinol complex (DHQ) on ovarian cancer cells, and the mechanism of action involved.
Methods: DHQ-mediated changes in cell viability were determined using MTT assay, while apoptosis was analyzed with flow cytometry. The effect of DHQ on cell migration was determined using inverted microscopy, while its effect on invasiveness was assessed with Giemsa dyeing. FACS Calibur instrumentation was employed for analyzing the effect of DHQ on the cell cycle. The protein expressions of Wip1 and P53 were assayed by western blotting.
Results: DHQ induced cytotoxicity against A2780 and OVCAR 3 cells in the concentration range of 0.25 - 12 µM (p < 0.05). In A2780 and OVCAR 3 cells, treatment with 12 µMDHQ resulted in 69.34 and 65.46 % apoptosis, respectively. The migratory potential and invasiveness of A2780 and OVCAR3 cells were significantly decreased by 12 µMDHQ, relative to untreated cells (p < 0.05). Moreover, treatment with 12 µMDHQ arrested cell cycle at G1/G0 phase in A2780 and OVCAR3 cells, but downregulated the protein expressions of Wip1 expression in A2780 and OVCAR3 cells.
Conclusion: DHQ exerts cytotoxic effect on ovarian cancer cell growth via arrest of cell cycle and activation of apoptosis. Moreover, DHQ inhibits the migratory and invasive abilities of the cells. Thus, DHQ is a potential drug candidate for the management of ovarian cancer.

Keywords: 5,7-Dihalo-8-quinolinol complex, Ovarian cancer, Cytotoxicity, Apoptosis, Invasiveness, Migration, Cell cycle

INTRODUCTION

Ovarian carcinoma is one of the most lethal malignancies and the 5th highest cause of cancer-related deaths in the United States [1]. The high mortality of ovarian cancer is associated with resistance to chemotherapy, tumor recurrence and elevated metastatic potential [2]. Moreover, up to 75 % of ovary cancer is diagnosed at the advanced stage, resulting in high mortality and poor prognosis [3]. Slowly-growing ovarian cancers detected at initial stage are known as type I tumors [4]. In contrast, the type II cancers which are highly aggressive, are detected at advanced stage and they grow very rapidly [4].

The common treatment for ovarian cancer is surgical resection aimed at decreasing the cancerous cells, and administration of taxol/platinum-containing chemotherapeutic agents. Although about 80% of ovarian cancer patients show good responses initially, tumor relapse has been reported in more than 50% of cases [4,5]. Patients who develop resistance to platinum or taxol are given more potent drugs, an example of which is topotecan [5]. Most ovarian cancer cells escape chemotherapeutic drugs by remaining in the non-proliferating and dormant stage [6].

These dormant cancerous cells start proliferating when the effect of the chemotherapeutic agent subsides, leading to tumor relapse and failure of therapeutic strategy [6]. Thus, there is a need for novel and effective therapeutic agents for treatment of ovarian cancer.

Induction of wild-type p53-induced phosphatase (Wip1) is generally observed in cells in the presence of ionizing radiations [7]. The Wip1 encodes a protein known as protein phosphatase magnesium-dependent 1 δ located in 17q22/24 region of chromosome in humans [7]. It belongs to the phosphatase type-2C (PP2C) family of proteins. Studies have reported Wip1 overexpression in different types of cancers and its association with carcinoma growth [8,9]. Therefore, Wip1 is considered a biomarker in various cancers, and it may act as target for therapeutic agents [8, 9]. Potent anti-tumor complexes were discovered by linking metal ions to organic compounds isolated from diversity of sources [10]. The improved pharmacological potential and limited side effects have encouraged clinicians to investigate the anti-tumor properties of other organo-metallic complexes [11]. This has led to identification of metal complexes which are effective in the inhibition of tumor growth at low doses, with minimum harmful effects on normal cells [12,13].

In the present study, the anti-cancer effect of DHQ on ovarian cancer cells was investigated.

EXPERIMENTAL

Cell lines and culture conditions

The A2780 and OVCAR 3 ovarian carcinoma cell lines were provided by the Cell Resource Center of Peking Union Medical College (Beijing, China). The cell lines were cultured for 24 h under 5% CO_2 atmosphere in DMEM at 37˚C. The medium contained 10% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL).

Assay of cell viability

The effect of DHQ on cell viability was determined using MTT assay. The cells were seeded in 96-well plates, each at a density of 1 x 10^6 cells/well, and cultured at 37˚C for 24 h. Then, DHQ was added to separate wells at concentrations of 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 12 μM, followed by incubation for 48 h. Thereafter, MTT solution (5 mg/mL) was added to the well plates, and incubation was continued for 4 h under similar conditions.

Following decantation of medium, DMSO (100 μL) was added to each well to solubilize the resultant formazan crystals. The absorbance of each well was read in an ELISA plate reader at 568 nm, and the readings were used to estimate the effect of DHQ on cell viability, relative to untreated cells.

Apoptosis assay

Cells treated with 12 μM DHQ and untreated cells were harvested, rinsed in PBS and subsequently fixed in 70% ethyl alcohol. Cells (at a density 1 x 10^5 cells/mL) were treated for 20 min with Annexin V-FITC (20 μL) at room temperature in the dark. Then, the cells were suspended in binding Buffer-1X and treated with propidium iodide for 4 min, also in the dark. Thereafter, cell apoptosis was analyzed in a flow cytometer (FACSCalibur, BD) with FlowJo software (FlowJo, BD).

Wound healing assay

The cells were seeded in6-well plates at a density of 1 x 10^5 cells/well, and maintained at 37 °C until 100% confluent cellular monolayers were obtained. A24-h cellular starvation was followed by creating a wound on the center of wells using a 100-ml pipette tip. The cells were treated with 12 μM DHQ or normal saline for 48 h, followed by washing with PBS at 37˚C. Thereafter, the cells were fixed in3.5% ethyl alcohol and subsequentlydyedwith2% crystal violet stain for 25 min. Cell migration was visualized and analyzed using a microscope (Nikon Corporation) in5 randomly-selected fields.

Invasion assays

The effect of DHQ on cellular invasiveness was determined in 24-well Transwell chambers (8-μm pore size Y). Matrigel (85 μg/ml; BD Biosciences) was applied onto the upper membrane surface and kept overnight at 37 °C. The cells were distributed in the upper chamber at a concentration of 1 × 10^5 cells/mL in 320 μl of

DMEM containing 10 % FBS, while 550 µL of DMEM containing 5 % FBS was put in the lower compartment. Then, the cells were cultured at 37 °C in an atmosphere of 5% CO_2 and 95% air for 48 h. The invaded cells were subjected to Giemsa dyeing (Sigma, USA), followed by analysis under a 200X phase-contrast microscope.

Cell cycle analysis

The cells were seeded at a density of 2 x 10^6 cells/well in 60-mm plates, and were treated with 12 µM DHQ or normal saline at 37°C for 48 h. Trypsinization and washing in PBS were followed by fixing in 70% ethyl alcohol overnight at 37°C. Then, the cells were treated with RNase A (20 µg/mL), and stained with PI (10 µg/mL) at 37°C. Thereafter, the distribution of cellular DNA content was determined using FACSCalibur instrumentation (BD Biosciences, San Jose, CA, USA).

Western blot analysis

Cells treated with 12 µM DHQ and untreated cells were harvested, rinsed in ice-cold PBS and subsequently subjected to lysis using RIPA buffer containing $PhCH_2SO_2F$ at 4°C for 30 min. The lysate was centrifuged at 12,000 x g for 15 min at 4°C, and the protein content of the supernatant was estimated using BCA protein assay kit. Then, equal amounts of proteins were separated on 10% SDS PAGE and transferred to PVDF membranes which were blocked by treatment with non-fat dry milk (5%) for 2 h. Protein probing was done on incubation of the membranes with primary antibodies against Wip1, P53 and GAPDH overnight at 4 °C.

Then, the membranes were washed with PBS, followed by incubation with horse radish peroxidase-conjugated goat anti-rabbit secondary antibody for 1 h at room temperature. The protein bands were detected using Bio-Rad ChemiDoc™ (Bio-Rad Laboratories, Co., Ltd, Hercules, CA, USA).

Statistical analysis

Data are presented as mean ± SD; differences between and amongst various groups were determined using Student *t*-test and one-way analysis of variance (ANOVA), respectively. Statistical analysis was carried out with GraphPad Prism-7 software (GraphPad Software Inc., La Jolla, CA, USA). Differences were taken as statistically significant at $p < 0.05$.

RESULTS

Inhibitory effect of DHQ on the viability of A2780 and OVCAR 3 cells

The cytotoxic effects of DHQ on A2780 and OVCAR 3 cells were measured at different durations using MTT assay. The viability of A2780 and OVCAR 3 cells were reduced to 47 and 51%, respectively, on treatment with 12 µM DHQ at 24 h (Figure 1 A). Treatment with 12 µM DHQ for 48 h suppressed viabilities of A2780 and OVCAR cells to 20 and 23 %, respectively (Figure 1 B). The changes in cellular viabilities due to 12 µM DHQ at 48 h were also determined using inverted microscopy (Figure 1 C). There were clear reductions in viabilities of the two cells treated with 12 µM DHQ for 48 h.
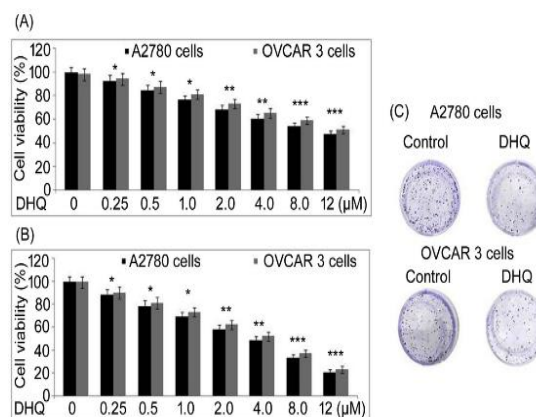

Figure 1: Effect of DHQ on cell viability. (A) Treatment of cells with 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 12 µM DHQ for 24 h was followed by determination of viability. (B) Exposure of the cells to 0.25, 0.5, 1.0, 2.0, 4.0, 8.0 and 12 µM DHQ for 48 h was followed by viability measurement. (C) Cell viability under an inverted microscope. *$P < 0.05$; **$p < 0.02$; ***$p < 0.01$ vs untreated cells

DHQ induced apoptosis in A2780 and OVCAR 3cells

The effect of DHQ on apoptotic changes in the cells were assayed using flow cytometry (Figure 2). Treatment with 12 µM DHQ for 48 h significantly increased apoptotic cell fractions in A2780 and OVCAR 3 cells, relative to control cells ($p < 0.05$). In A2780 cells, treatment with 12 µM DHQ for 48 h resulted in 69.34 % apoptosis, relative to 2.14 % apoptosis in control cells. Similarly, treatment of OVCAR-3 cells with 12 µM DHQ for 48 h produced 65.46% apoptosis, which was significantly higher than 1.78 % apoptosis in control cells.
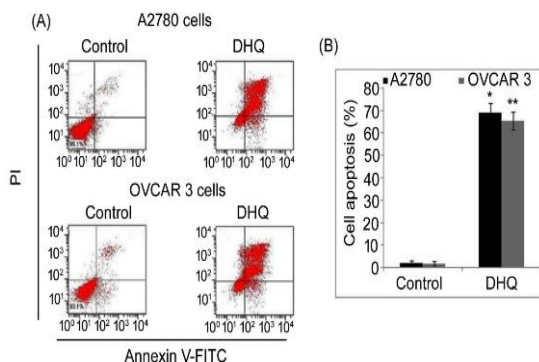
Figure 2: Effect of DHQ on apoptosis of A2780 and OVCAR 3 cells. Treated and untreated cells were analyzed for apoptosis at 48 h after Annexin V-FITC/PI staining using flow cytometry. *$P < 0.02$; **$p < 0.01$, vs untreated cells

DHQ inhibited cell migration

The effect of DHQ on cell migration was determined using wound healing assay. As shown in Figure 3, the migratory potential of the cells was significantly decreased by treatment with 12 µM DHQ, when compared with untreated cells. In A2780 cells, treatment with 12 µM DHQ decreased the migrating ability to 21.56 %, relative to 98.34 % in untreated control. The DHQ treatment also suppressed OVCAR 3 cell migrating ability to 23.87%, relative to 98.21 % in untreated cells.
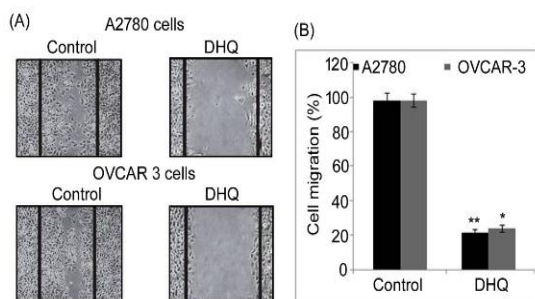

Figure 3: Effect of DHQ on migrating ability of the cells. The cell migration abilities of A2780 and OVCAR 3 cells treated with 12 µM DHQ were determined with light microscopy. *$P<0.02$; **$p<0.01$ vs. untreated cells. Magnification, x250

DHQ suppressed cell invasiveness

Wound healing assay was used to determine the effect of DHQ on cell invasive abilities. As shown in Figure 4, the invasiveness of cells treated with 12 µM DHQ was significantly decreased, when compared to untreated cells. In A2780 cells, treatment with 12 µM DHQ decreased invasive ability to 28.22 %, relative to 97.98 % for untreated control. Similarly, the 12 µM DHQ

treatment suppressed OVCAR 3 cell migrating ability to 29.88 %, relative to 99.10% for untreated cells.
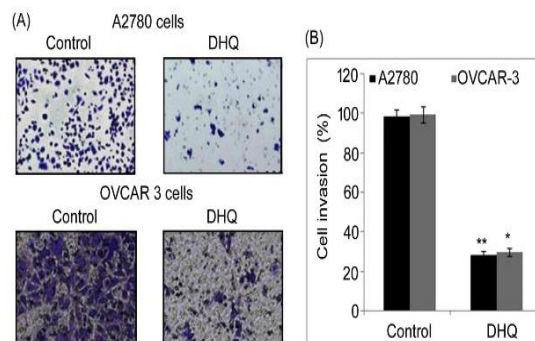

Figure 4: Effect of DHQ on invasiveness of the cells. Cell invasion potential of A2780 and OVCAR 3 cells on treatment with12 µM DHQ were assayed using Transwell chambers. *$P<0.02$; **$p<0.01$ vs. untreated cells. Magnification, x250

DHQ caused arrest of cell cycle atG1/G0 phase

The effect of DHQ on the cell cycle was determined using flow cytometry (Figure 5). Treatment of the cells with 12 µM DHQ significantly enhanced the population of cells at the G1/G0 phase, relative to untreated cells. There were significant reductions in cell content at the S and G2/M phases in A2780 and OVCAR 3 cells treated with 12 µM DHQ for 48 h. Thus, DHQ treatment caused arrest of cell cycle at the G1/G0 phase.
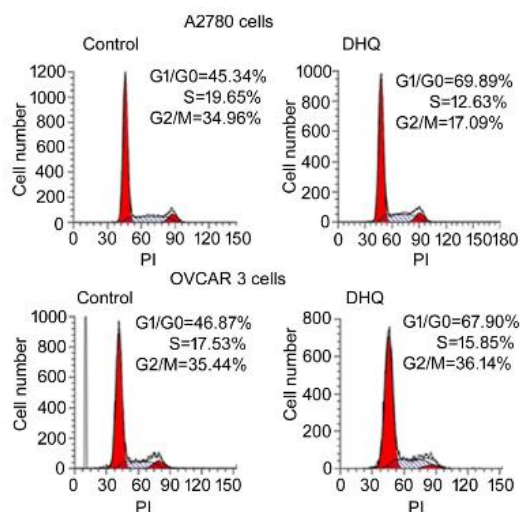

Figure 5: Effect of DHQ on the cell cycle. Cells treated with 12 µM DHQ and untreated controls were analyzed for changes in cellular DNA contents using flow cytometry after PI staining

DHQ suppressed the expression of Wip1 in A2780 and OVCAR cells

The expression of Wip1 was markedly down-regulated in the cells by treatment with 12 µM DHQ (Figure 6). In DHQ-treated cells, markedly lower Wip1 mRNA expression, relative to control cells was observed. The DHQ-mediated suppression of Wip1 protein expressions in A2780 and OVCAR 3 cells were also confirmed using western blotting.
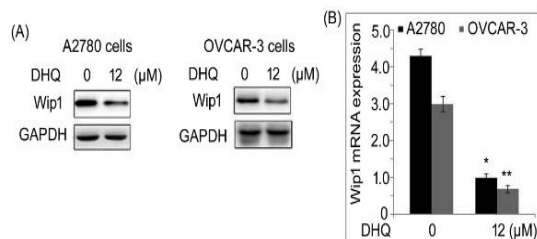

Figure 6: Effect of DHQ on Wip1 expression in the cells. (A) Cells treated with 12 µM DHQ and untreated cells were analyzed for Wip1 mRNA expression. (B) Changes in Wip1 protein expression in untreated or 12 µM DHQ-treated cells, as assayed using Western blotting. $*P < 0.02$, $**p < 0.01$ vs untreated cells

DISCUSSION

Cancer is a worldwide health problem that accounts for 13 % of mortality globally [3]. It has been projected that mortalities associated with cancer will increase to13.2million by the year 2030, relative to about 7.5 million deaths at present [3]. Non-functional and damaged cells in multi-cellular organisms are removed via the apoptotic pathway [14]. Chemotherapeutic agents suppress carcinoma growth by inducing DNA damage, followed by activation of apoptosis [15]. Apoptotic cells undergo shrinkage in size, chromatin condensation, formation of blebs, and cleavage of DNA [14]. In the current study, DHQ exerted toxicity on ovary carcinoma cells in a dose-depending manner. The DHQ treatment significantly reduced cellular viabilities in the ovarian cancer cells, relative to untreated cells. The treatment enhanced apoptosis activation, which was evident in the higher apoptotic cellular fraction in DHQ-treated cells. Moreover, DHQ reduced the migratory and invasive abilities of the cells, relative to control cells.

The oncogene, Wip1 which was identified in the nineteenth century, has been investigated by various research groups with respect to its involvement in different cancers [16]. It deactivates multiple proteins which are key components of DNA damage repair pathways [17]. The deactivation of some of these proteins,

for example check point kinase-1, p53 and p16, causes tumorigenesis [17]. Thus, Wip1 is the negative regulator of DNA damage repair pathway, and an inducer of tumorigenesis [17]. In contrast, p53 is a tumor suppressor factor actively involved in repair of DNA damage via regulation of cellular apoptosis [18]. Thus, p53 dysfunction increases the resistance of tumors to therapeutic agents [19]. Studies have revealed that Wip1 mediated inhibition of p53 protein either by direct dephosphorylation or via inactivation of p38, MAPK and Mdm2 [20, 21]. Thus, the regulation of Wip1 expression so as to restore p53 activity has therapeutic role in various cancers.

In many solid tumors such as hepatic cancer, nasopharyngeal carcinoma, pulmonary cancer and prostate carcinoma, poor prognosis is related to increased Wip1 expression [22, 23]. The present study revealed elevated expressions of Wip1 (mRNA and protein) in the ovarian cancer cells. However, DHQ treatment significantly down-regulated the RNA and protein expressions of Wip1 in A2780 and OVCAR 3 cells, relative to control.

CONCLUSION

The findings of this study have shown that DHQ exerted cytotoxic effects on ovarian cancer cell growth via arrest of cell cycle and activation of apoptosis. Moreover, DHQ inhibits the migratory and invasive ability of A2780 and OVCAR 3 cells. DHQ-mediated cytotoxic effect occurs via a mechanism associated with down-regulation of mRNA and protein expressions of Wip1. Therefore, DHQ has potentials for use in the treatment of ovarian cancer.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Han Qi - conceived and designed the study; Rao Zhiwei, Xia Songbai collected and analyzed the data; Rao Zhiwei, Han Qiwrote the manuscript. All authors read and approved the manuscript for publication.

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