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short report

Effects of Sulforaphane and Cisplatin Treatments on A2780-CP20 Ovarian Cancer Cell Line

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Abstract

Sulforaphane is an active component found in cruciferous vegetables, such as broccoli, that has been shown to exhibit anticancer properties. For example, in a breast cancer model, sulforaphane reduced the viability of breast cancer cells. Moreover, cisplatin is an anticancer drug used to treat ovarian cancer. However, the numerous side effects of cisplatin can be unbearable and some ovarian cancers are resistant to cisplatin treatment. Therefore, identifying the effects of sulforaphane on ovarian cancer cells will potentially lead to a better strategy. In this study, we determine the effects of sulforaphane, cisplatin and a combination of sulforaphane and cisplatin treatment on the cell proliferation and morphology of A2780-CP20 cancer cells that are cisplatin resistant. Results of this study show that sulforaphane is a good component that also targets growth of ovarian cancer cells. The overall trend from the data gathered from cell viability assay, cell morphology analysis, and colony forming assay showed A2780-CP20 cells are dose-dependent with respect to viability and morphology. Further experiments can now be performed to determine the effects of these compounds on ovarian cancer cell proliferation and to understand the mechanisms involved.

Introduction

Ovarian cancer is the uncontrollable growth of cells near the ovaries—as there have been indications of possible extra ovarian sites in the fallopian tubes—that causes older women to experience pain in the lower abdomen[3]. Lack of treatment or early diagnosis of ovarian cancer can result in a premature death. According to Centers for Disease Control and Prevention, in 2007, approximately 20,749 women in the United States were diagnosed with ovarian cancer of which 14, 621 died[1]. (It is to be noted that the diagnosed women are not the same who died in 2007). Current primary treatment regimes second to surgery for ovarian cancer include chemotherapy with cisplatin.

Cisplatin is an anti-cancer drug that binds to the DNA of cancerous cells and inhibits the cell cycle, causing reduced growth and apoptosis (Figure 1)[3]. Although cisplatin provides a treatment mechanism for ovarian cancer, the side effects of the drug are a major problem for a subset of patients because the drug does not differentiate targeting between normal and cancerous cells. Chemotherapy with cisplatin includes, but is not limited to the following symptoms: hair loss, suppression of the immune system, and nausea[2]. As such, it can be concluded for a subset of patients with significant side effects, cisplatin must be prematurely terminated making it unsatisfactory as a mono-therapy[3].

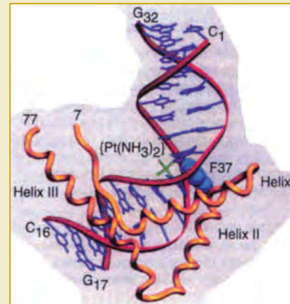

*Figure 1: Cisplatin binding of the DNA of cancerous cells is depicted. Cisplatin molecules are the small blue stick models bound to the DNA double helix.**

Research findings have indicated that sulforaphane (SFN), a dietary compound found in cruciferous vegetables and a potential chemotherapy drug, can be used to treat and prevent breast cancer[6]. For example, as seen in Figure 2, SFN has been shown to regulate histone-modifying enzymes, resulting in a relaxed chromatin structure that allows for a more direct access to DNA[5]. Also, in a previous breast cancer model, SFN decreased the viability of cells[5].

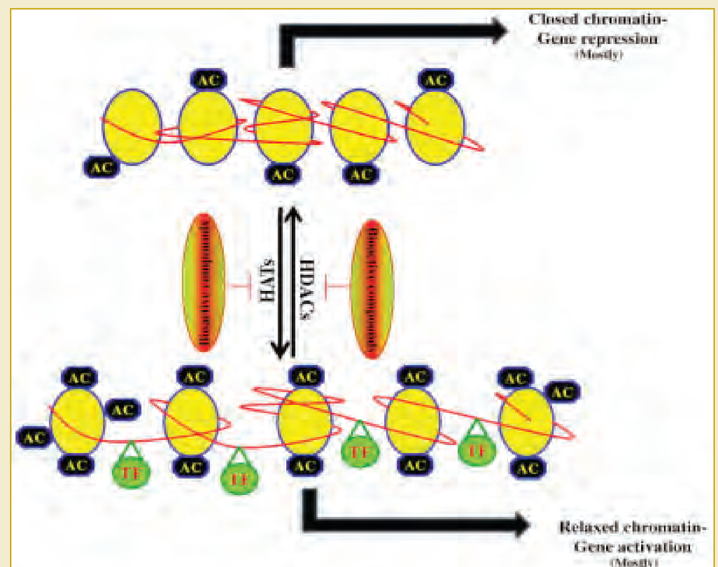

*Figure 2: Histone modifications caused by SFN as found in preliminary data. Figure has been linked to show SFN's anti-cancer properties.***

The purpose of this research is to determine the effects of sulforaphane on the A2780-CP20 ovarian cancer cell line. The A2780-CP20 cell line is estrogen receptor-negative, a subtype of cancerous cells. An estrogen receptor-negative cell line lacks the expression of estrogen receptors on the cells[9]. As such, estrogen receptor-negative cancer cells do not respond to hormonal treatments that are receptor-targeted, making the cancer more difficult to treat[9]. Nonetheless, some ovarian can

cer cells have been shown to respond to chemotherapy drugs such as cisplatin that do not target hormonal receptors as another mode of treatment[9]. Unfortunately, clinically treatment has shown A2780-CP20 to be resistant to cisplatin treatment. However, it is to be noted that the cisplatin-resistant nature of the cell line is not an all-or-none phenomena. Cisplatin resistance is based on many factors, including "cisplatin uptake and efflux, inactivation by thiol-containing molecules, or could be caused by the sensitivity of the target cell to DNA damage caused by cisplatin"[3]. If the effects of sulforaphane can be thoroughly understood with a combination treatment with cisplatin, a more natural treatment method can be used to treat cisplatin-resistant and estrogen receptor-negative ovarian cancer.

Methods and Materials

Cell Culture

A2780-CP20 estrogen receptor-negative, cisplatin resistant human ovarian cancer cell line was cultured in 37°C and 5% CO_2 with RPMI medium supplemented with 10% Fetal Bovine Serum and 1% penicillin/streptomycin[8].

MTT Cell Viability Assay

A MTT cell viability assay was performed to determine the effects of sulforaphane, cisplatin, and a combination treatment of the two drugs on the cell viability of the ovarian cells. Initially, a cell count was tabulated using a hemacytometer (ratio of cell to trypan blue stain 1:1). A total of 10,000 cells per well were plated for each dose. The cells were allowed to adhere onto the wells of microtiter plate for 24 hours and then were treated with increasing concentrations of sulforaphane (0, 1.0, 2.5, 5.0, 10.0, and 20.0 μM) and cisplatin (0, 1.0, 1.5, 3.0, and 10.0 μM). The combination treatment included the following concentrations of sulforaphane and cisplatin, respectively: 1.0 + 1.5, 2.5 + 1.5, 5.0 + 1.5, 1.0 + 3.0, 2.5 + 3.0, and 5.0 + 3.0 μM. Cell viability was measured after 72 hours of treatment using a microplate reader to detect MTT absorbance of the living cells.

A 50 μL working stock MTT (50 μg/mL) was added to each well and incubated for approximately 2 hours. Then 150 μL dimethyl sulfoxide (DMSO) was added to each well to dissolve the formazan precipitate formed by the MTT. The microtiter plate was shaken for 10 minutes and the absorbance of MTT due to the purple color change of the cells was read using the microplate reader in a stepwise function at 540 nm wavelength with a reference wavelength of 650 nm.

Colony Forming Potential Assay

A colony assay was utilized to view the effects of sulforaphane, cisplatin, and a combination treatment on the potential for these cells to form colonies and confirm results gathered from MTT assay[7]. As cells tend to grow in colonies, the ability to form a colony suggests the way the cells grow was not affected by treatment. This assay was different from the cell viability assay as it was used to more readily see the cell growth, not quantitatively like in the case of the cell viability assay, but qualitatively. The colony forming assay's main purpose is to view changes in cell proliferation.

The most ideal and effective doses shown by MTT data were used in the colony formation assay. A cell count was determined using aforementioned method. Using a 6-well cell culture plate, 500 cells were adhered onto each well for 15 days after treatment with sulforaphane, cisplatin, and a combination of the two substances. The sulforaphane concentrations used to treat the cells were 0, 1.0, 2.5, 5.0, 10.0, and 20.0 μM. The cisplatin concentrations were 0, 1.0, 1.5, and 3.0 μM. The combination treatment included the following concentrations of sulforaphane and cisplatin, respectively: 0, 1.0 + 1.5, 2.5 + 1.5, 5.0 + 1.5, 1.0 + 3.0, 2.5 + 3.0, and 5.0 + 3.0 μM. Cell colony formation was measured after the 15 day treatment period by fixing the cells with 70% ethanol and staining the colonies formed with a trypan blue solution. The number of cell colonies (>50 cells/colony) that remained viable were counted.

Cell Morphology Analysis

Cell morphology analysis was performed to view the effects of drug treatment on differences in cell shape. A cell count with a hemocytometer was performed to determine the exact number of cells to pipette into a plate. Equal number of cells $1X10^5$ cells per plate were added to 12 separate culture dishes in order to view the effects of 12 different drug treatments with sulforaphane, cisplatin, and combination treatment. The doses used were obtained from MTT data and were as follows: sulforaphane (0, 2.5, 5.0, 10.0 μM), cisplatin (0, 1.5, 3.0, 10.0 μM), and combination of sulforaphane and cisplatin, respectively (0, 2.5 + 1.5, 5.0 + 1.5, and 2.5 + 3.0 μM). After a 72 hours treatment period, pictures of cell shape were taken at 100X magnification using a camera. The pictures that were selected best represented the overall image of the cells with the most clarity.

Results

In order to characterize the effects of sulforaphane and cisplatin treatment, a 72 h MTT assay was performed and all drug treatments were compared to the untreated control (Figure 3). Treatment with sulforaphane and cisplatin showed a dose-dependent trend in cell viability: as the drug concentration increased, the drug toxicity to the cells also increased. For the combination treatment, however, a doses-dependent response was not observed. Individual sulforaphane and cisplatin optimum doses, respectively, are 20 and 10 μM. The optimal combination dose was 5.0 + 1.5 μM.

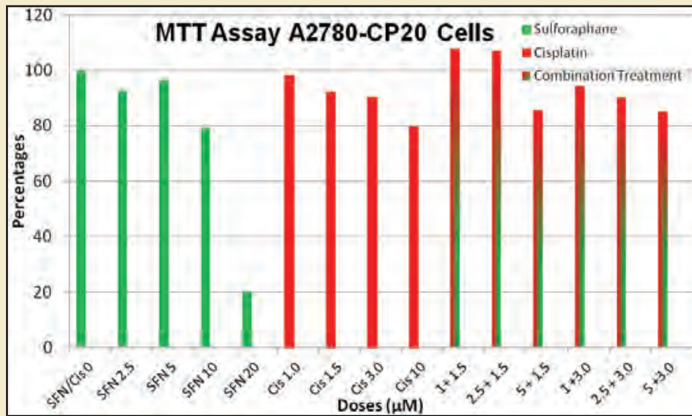

Figure 3: MTT cell viability assay (72 h) showed that sulforaphane and cisplatin have a dose-dependent trend in cell viability; combination treatment of the drugs showed that the optimum doses of sulforaphane and cisplatin were 5.0 and 1.5 µM concentrations.

The results of the MTT analysis were confirmed with a colony forming assay (see Figure 4). The colony assay did not show as gradual of a dose-dependent result of the effect of sulforaphane on the cell line; rather, it showed a more drastic decrease from SFN 5 µM to SFN 10 µM. Also, this experiment and the replicate were only conducted once for preliminary data gathering and does need to be repeated in order to confirm the results shown below.

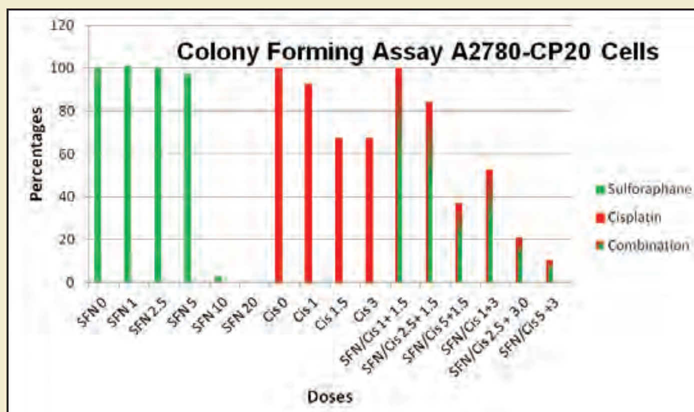

Figure 4: Colony forming assay showed that the colony also has a dose-dependent response to drug treatment.

It was predicted that the drug treatments may affect the morphology of the A2780-CP20 cell line. As such, a cell morphology analysis was performed in which the cells were treated with doses found effective by the MTT and colony forming assays (Figure 3). With increasing concentration, the cell growth was visibly shown to be inhibited for the individual doses of sulforaphane and cisplatin. However, the combination treatments did not show as much response to changes in cell morphology or the physical shape of the cells.

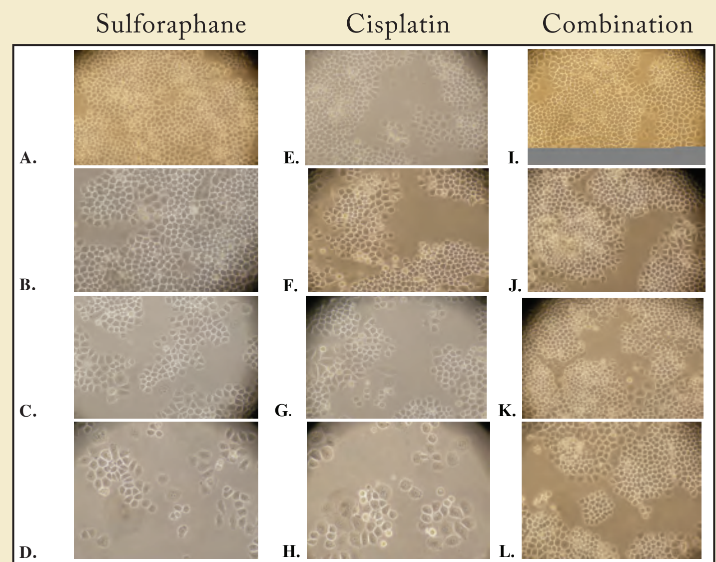

Figure 5: Cell Morphology Analysis of the Effects of Sulforaphane, Cisplatin, and Combination Treatment. Photos A, E, and I show untreated A2780-CP20 cells for sulforaphane, cisplatin, and combination, respectively. Photos B-D show the following sulforaphane dosages respectively: 2.5, 5.0, and 10.0 µM. Photos F-H show the following cisplatin dosages respectively: 1.5, 3.0, and 10.0 µM. Photos J-L show the following combination dosages: 2.5 (sulforaphane) + 1.5 (cisplatin), 5.0 (sulforaphane) + 1.5 (cisplatin), and 2.5 (sulforaphane) + 3.0 (cisplatin) µM.

Discussion

Previous studies with a breast cancer model have shown that sulforaphane causes a decrease in cell proliferation[5]. It was predicted that sulforaphane may yield similar results in an ovarian cancer model. The MTT analysis showed that sulforaphane produces a dose-dependent yield in estrogen receptor-negative ovarian cancer cells with the optimum dose observed to be 20 µM. Cisplatin showed a similar trend with an optimum dose of 10 µM; however, during the combination treatment the optimum dose was found to be 5.0 µM sulforaphane and 1.5 µM cisplatin. As stated earlier, cisplatin treatment is not an all-or-none phenomenon. Within the cell line certain cells showed less resistance to the treatment than others, yielding a dose-dependent response. Unlike the individual doses, a dose-dependent response was not observed with the combination treatments for MTT. The results led us to believe that the two drugs may not be working together to have a combined effect.

The colony forming assay generally confirmed the results shown by the MTT analysis; however, the colony forming assay did not show a gradual dose-dependent trend with sulforaphane treatments as did the MTT analysis. A more drastic decrease is noticed between sulforaphane treatments of 5 and 10 µM. Also, the colony results shown are from one trial; therefore the assay needs to be repeated several times to confirm the results.

The morphological affects of the drug treatments were viewed with the cell morphology assay. As the MTT analysis suggested, the increase in dosages of sulforaphane and cisplatin led to a decrease in cell viability. Optimum doses of both sulforaphane and cisplatin were concluded to be 10 μM. The morphology analysis suggested that the optimum combination dose is 2.5 (sulforaphane) + 3.0 (cisplatin) μM. No change in cell morphology was observed; however, a dose dependent decrease in cell proliferation was noticed.

Collectively, the data showed a dose-dependent response for the individual treatments but not a combination treatment. It is possible that either the sulforaphane or cisplatin alone may be the driving force for the slight decrease in cell viability observed with the combination treatments. Statistical analysis still needs to be performed to determine the degree of significant change in cell proliferation. In addition, sulforaphane treatments of A2780-CP20 cells shows promise for decreasing the growth of ovarian cancer cells, leading to the possibility of using a natural approach to chemoprevention. With neglible to no sideeffects from sulforaphane treatment, this natural approach would be chosen over the cisplatin treatment clinically. To ensure the lack of sulforaphane toxicity to normal cells, this study needs to be performed on normal ovarian HIO-180 cells in the future.

Conclusion

Finding a better treatment method for ovarian cancer cells can allow for more effective treatment with less deterring side effects. Sulforaphane is a natural component with anticancer properties that would help provide a treatment regime for cancer cells, as it has been shown to diminish cell viability. Results of this study show that sulforaphane is a good component that also targets growth of ovarian cancer cells. As such, further study and data analysis needs to be completed in order to fully understand the biological mechanisms that cause the affects of sulforaphane on cell viability in an ovarian cancer model.

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