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BRIEF COMMUNICATION

Hymenialdisine is Cytotoxic Against Cisplatin-Sensitive but Not Against Cisplatin-Resistant Cell Lines

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abstract: ***Objectives:*** New compounds are needed to overcome the resistance to commonly used cytotoxic chemotherapy for epithelial ovarian cancer. Marine sponges are a rich source of diverse chemical compounds and hymenialdisine has been found to have antiproliferative effects. This study aimed to investigate the cytotoxic effect of hymenialdisine in cisplatin-sensitive and cisplatin resistant ovarian cancer cell lines. ***Methods:*** This study took place at Sultan Qaboos University, Muscat, Oman between August and November, 2019. The anti-cancer effects of hymenialdisine or cisplatin were assessed using treating cells with different concentrations of hymenialdisine and cisplatin. Cell viability was determined using the AlamarBlue® Assay. ***Results:*** The half-maximal inhibitory concentration (IC50) of cisplatin was estimated at 31.4 µM for A2780S and 76.9 µM for A2780CP, whereas the IC50 of hymenialdisine was evaluated at 146.8 µM for A2780S cells. Despite the higher concentrations of hymenialdisine (up to 300 µM), IC50 could not be determined for the A2780CP cell line. ***Conclusion:*** When compared to cisplatin, hymenialdisine was less toxic against both A2780S and A2780CP ovarian cancer cell lines.

Keywords: Gynecologic Neoplasm; Ovarian Cancer; Cisplatin; Hymenialdisine; Oman.

Epithelial ovarian cancer (EOC) is one of the most common gynaecological cancers and a leading cause of cancer-related deaths.[1] Combination chemotherapy consisting of cisplatin and paclitaxel has been the standard of care; however, the vast majority of patients develop resistance, especially to the platinum drug.[2,3] The five-year survival remains dismally low at 15–25%, and therefore, new compounds are needed to achieve better disease control and survival.[4] In the previous study by Dobretsov *et al*., hymenialdisine was observed to have potent cytotoxic activity against the Michigan Cancer Foundation-7 breast cancer cell line.[5] Hymenialdisine is a marine alkaloid consisting of a pyrrole-ring fused to an azepine that was first isolated from the marine sponges *Acanthella* sp. and *Axinella* sp. in 1982.[6] It has been shown to be a potent inhibitor of a number of kinases and proteins that regulate membrane transport, gene expression, cellular proliferation and apoptosis. This study investigated the cytotoxic effect of hymenialdisine in both cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines to determine whether the compound would be effective against ovarian cancer cells.

Methods

This study was conducted at Sultan Qaboos University, Muscat, Oman between August and November, 2019. Hymenialdisine (Boc Science, New York, USA) was dissolved in dimethyl sulfoxide (Sigma-Aldrich, Burlington, Massachusetts, USA) to create a stock solution of 3085.2 µM. Cisplatin solution (1 mg/mL; Mylan S.A.S, Saint Priest, France) was obtained at a concentration of 3333.2 µM. Cisplatin-sensitive (A2780S) and cisplatin-resistant (A2780CP) ovarian cancer cell lines.

The cell lines were seeded in a 96-well plate, cultured in Dulbecco's Modified Eagle's Medium (DMEM/F12) and maintained as has been described earlier.7 The anti-cancer effects of hymenialdisine and cisplatin were assessed by treating cells with different concentrations of hymenialdisine (100–300 µg/mL) or cisplatin (10–70 µM) over a 24-hour period. Cell viability was determined using the AlamarBlue® Assay (Invitrogen, Waltham, USA) and following the manufacturer's protocol. Briefly, the cells were stained after trypsinisation by trypan blue and counted. Approximately 20,000 cells were grown to confluence in a complete growth medium (DMEM/F12 + 10% fetal bovine serum). Then, cells were exposed to different concentrations of hymenialdisine and cisplatin in serum-free media. AlamarBlue® (Invitrogen) was added to the cells in an amount equal to 10% of the final volume in the well 24 hours after the treatment.

Three hours later, the absorbance was measured at 570 and 600 nm using Thermo Multiskan Spectrum Spectrophotometer (Thermo Fisher Scientific,

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Waltham, USA). One-way analysis of variance, followed by Tukey's post-hoc test, was used to determine whether there was any difference. P <0.05 was considered statistically significant. Half-maximal inhibitory concentration (IC50) was obtained using Graphpad Prism, Version 8.0.2 (GraphPad Software Inc, San Diego, California, USA) and Microsoft Office Excel, Version 2016 (Microsoft Corp., Redmond, Washington, USA). Results were expressed as mean ± standard deviation.

This study was approved by the institutional medical research committee.

Results

A2780CP was 2.5 times more resistant when treated with cisplatin compared to A2780S cancer cell lines. The viability of cells was reduced to <50% at a concentration of 300 µM of hymenialdisine, whereas no significant reduction in the viability of A2780CP was detected despite increasing the concentration of hymenialdisine to 300 µM. The IC50 of cisplatin was estimated at 31.4 µM for A2780S and 76.9 µM for A2780CP, whereas the IC50 of hymenialdisine was evaluated at 146.8 µM for A2780S cells. Despite the higher concentrations of hymenialdisine used (300 µg/ml), IC50 could not be calculated for the A2780CP cell line. All results are from three independent experiments for A2780S (P <0.0001) and A2780CP (P <0.0005) [Figure 1].

Discussion

The current study found that, when compared to cisplatin, hymenialdisine was less toxic against both A2780S and A2780CP ovarian cancer cell lines. These results are different from our previous study, where hymenialdisine demonstrated portent cytotoxic action against the Michigan Cancer Foundation (MCF)-7 breast cancer cell line (IC50 at <100 µg/mL).[5] Several kinase inhibitors such as hymenialdisine have strong selectivity not only for specific kinase subtypes but also for cancer cells. Hymenialdisine in the micromolar range was shown to have antiproliferative effects against human tumor cell lines, exerting its antiproliferative activity through the inhibition of cyclin-dependent kinase (CDK)-1/cyclin-B, CDK-2/cyclin-A, mitogen-activated protein kinase-1, casein kinase 1, protein serine/threonine kinases CDK5, CDK-2/cyclin E, glycogen synthase kinase 3 and creatine kinase 1.[8,9] These kinases and transcription factors are vital for processes such as cellular proliferation, gene expression and apoptosis. On the other hand, several other enzymes are only modestly inhibited by hymenialdisine, such as 3' 5'-cyclic adenosine monophosphate-dependent protein kinase, insulin receptor tyrosine kinase, c-Src tyrosine kinase and c-Abl tyrosine kinase.[10]

The results of the current study clearly demonstrated the different responses of cisplatin-sensitive and cisplatin resistant cell lines to cisplatin. The resistant cell line was at least 2.5 times more resistant compared to the sensitive cell line. The mechanisms of cisplatin resistance include the activity of general efflux mechanisms, post-translational modification, a defect in the mismatch repair pathway due to the silencing of the *human mutL homolog 1* gene and expression of small non-coding RNAs.[11–13] However, the reason for resistance against hymenialdisine remains speculative. One possible explanation is the differential expression of 11 miRNAs in cisplatin-resistant cells, which have targets in the transforming growth factor-B and mitogen-activated protein kinase (MAPK) signalling pathways.[14] The activation of MAPK due to phosphorylation can lead to either cell proliferation or apoptosis. Aldisine

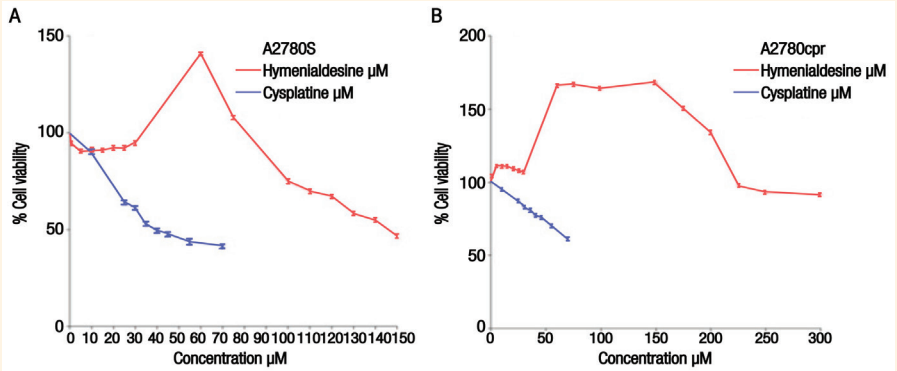

Figure 1: A: Viability percentage for A2780S in the presence of different concentrations of cisplatin (IC50 at 31.4 µM) and hymenialdisine (IC50 at 146.6 µM. **B:** Viability percentage for A2780CP in the presence of different concentrations of cisplatin (IC50 at 76.9 µM) and hymenialdisine (IC50 was not reached at 300 µM).

alkaloids, including hymenialdisine, which act through the inhibition of MAPK-1, may inhibit apoptosis and promote cell proliferation because of the differential expression of miRNAs in the cisplatin-resistant cells.[9] Alternatively, hymenialdisine may exert its anti-proliferative activity through the inhibition of membrane transport, cell proliferation and/or differentiation. The mechanisms through which drugs such as cisplatin and hymenialdisine interact need to be studied further.

Conclusion

While the results of this study showed the inhibitory effect of hymenialdisine against cisplatin sensitive cell lines, the effect against the cisplatin-resistant cell lines was not pronounced. This could have been either due to a similar mechanism of toxicity between cisplatin and hymenialdisine or due to hitherto unexplained mechanisms. Therefore, the study of the mechanism of action of hymenialdisine would be useful if the compound were to be used in combination with cytotoxic chemotherapy or used to overcome drug resistance.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTION

NA and NB carried out the experiments in the laboratory; SIH, SB, SD, YT and IB wee involved in designing of the experiments and in writing and reviewing the manuscript. All authors approved the final version of the manuscript.

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