Abstract
Introduction: 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. We investigated the cytotoxic effect of hymenialdisine in cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines. Methods: The anti-cancer effects of hymenialdisine or cisplatin were assessed by treating cells with different concentrations of hymenialdisine and cisplatin. Cell viability was determined using the AlamarBlue® Assay. Results: The IC\textsubscript{50} of cisplatin was estimated at 31.4 μM for A2780S and 76.9 μM for A2780CP, whereas the IC\textsubscript{50} of hymenialdisine was evaluated at 146.8 μM for A2780S cells. Despite the higher concentrations of hymenialdisine (up to 300 μM), IC\textsubscript{50} 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: Ovarian Cancer, Marine compounds, Cisplatin, Hymenialdisine, Oman
Introduction

Epithelial ovarian cancer (EOC) is one of the most common gynecological cancers and a leading cause of death from cancer\(^1\). Combination chemotherapy consisting of cisplatin and Paclitaxel has been the standard of care; however, the vast majority of patients develop resistance, especially to platinum drug\(^2,3\). The 5-year survival remains dismally low at 15-25\(^\%\)\(^4\), and—therefore, new compounds are needed to achieve better disease control and survival. In our previous study, hymenialdisine had potent cytotoxic activity against the MCF7 breast cancer cell line\(^5\). Hymenialdisine is a marine alkaloid consisting of a pyrrole ring fused to an azepine that 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. We investigated the cytotoxic effect of hymenialdisine in both cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines to see whether the compound would be effective against ovarian cancer cells or not.

Methods

Hymenialdisine was purchased from Boc Science (USA) and dissolved in dimethyl sulfoxide (DMSO) (Sigma, USA) to make a stock solution of 3085.2\(\mu\)M. Cisplatin solution (1 mg/ml) was obtained from Mylan, France, at a concentration of 3333.2\(\mu\)M. The cisplatin-sensitive (A2780S) -resistant (A2780CP) ovarian cancer cell lines were a generous gift of Dr. Benjamin K. Tsang (University of Ottawa, Ottawa, Canada). The cell lines were seeded in a 96-well plate and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM/F12) and maintained as described earlier\(^7\). The anti-cancer effects of hymenialdisine and cisplatin were assessed by treating cells with different concentrations (100-300 \(\mu\)g/ml) of hymenialdisine and (10-70 \(\mu\)M) of cisplatin for 24 hours. Cell viability was determined using the AlamarBlue\textsuperscript{\textregistered} Assay (Invitrogen, USA), using the manufacturer’s protocol. Briefly, the cells were stained after trypsinization by trypan blue and counted. Around 20,000 cells were grown to confluence in a complete growth medium, DMEM/F12 + 10\% fetal bovine serum (FBS). Then, cells were exposed to different concentrations of hymenialdisine and cisplatin in serum-free media. Twenty-four hours after treatment, AlamarBlue\textsuperscript{\textregistered} was added to the cells in an amount equal to 10\% of the final volume in the well.
Three hours later, the absorbance was measured at 570 and 600 nm using Thermo Multiskan Spectrum Spectrophotometer (Thermo Fisher Scientific, USA). One-Way ANOVA followed by Tukey’s post-hoc test was used to detect the difference, and a p < 0.05 was set as significant. Median inhibitory concentration IC_{50} was obtained by the Prism graph pad version 8.0.2 software and Microsoft Office Excel and expressed as the mean ± standard error. The study was approved by the institutional medical research committee.

**Results**

The effect of cisplatin on cell viability is summarized in Figure 1A; A2780CP was 2.5 times more resistant when treated with cisplatin and compared to A2780S cancer cell lines. The viability of cells was reduced to less than 50% at a concentration of 300 μM of hymenialdisine, whereas no significant reduction in viability of A2780CP was detected despite increasing concentrations of hymenialdisine to up to 300 μM of hymenialdisine, as shown in figure 1B. The IC_{50} of cisplatin was estimated at 31.4 μM for A2780S and 76.9 μM for A2780CP, whereas the IC_{50} of hymenialdisine was evaluated at 146.8 μM for A2780S cells. Despite the higher concentrations of hymenialdisine used (300 μg/ml), IC_{50} could not be calculated for the A2780CP cell line.

**Discussion**

When compared to cisplatin, hymenialdisine was less toxic against both A2780S and A2780CP ovarian cancer cell lines. These results are at variance with what was observed in the case of the MCF-7 breast cancer cell line^5^. Several kinase inhibitors such as hymenialdisine have strong selectivity not only for specific kinase subtypes but also for cancer cells. Hymenialdisine has been found to have antiproliferative effects against cultured lymphocytic leukemia cells (IC_{50} 18μg/ml) and LoVo adenocarcinoma cell line (IC_{50} 710 nM), and MCF-7 (IC_{50} <100μg/ml)^5. Hymenialdisine exerts its antiproliferative activity through inhibition of 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 (GSK-3), and creatine kinase 1 (CK1)^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 cAMP-dependent protein kinase, insulin receptor tyrosine kinase, c-src tyrosine kinase, and c-abl tyrosine kinase^10^. 
Our results clearly demonstrate 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 as compared to the sensitive cell line. The mechanisms of cisplatin resistance include the activity of general efflux mechanisms, post-translational modification, a defect in mismatch repair pathway due to the silencing of the MLH1 gene, and expression of small non-coding RNAs\textsuperscript{11-13}. 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 TGF-B and MAPK signaling pathways\textsuperscript{14}. Activation of MAPK due to phosphorylation can lead to either cell proliferation or apoptosis. Aldisine alkaloids, including hymenialdisine, which act through inhibition of MAPK-1, may inhibit apoptosis and promote cell proliferation because of the differential expression of miRNAs in the cisplatin-resistant cells\textsuperscript{9}. Alternatively, hymenialdisine may exert its anti-proliferative activity through inhibition of membrane transport, cell proliferation and/or differentiation. The mechanisms by which drugs such as cisplatin and hymenialdisine interact need to be studied further.

**Conclusion**
In summary, while our results showed the inhibitory effect of hymenialdisine against cisplatin-sensitive cell lines, the effect against the cisplatin-resistant cell lines was not pronounced. This may be either due to similar mechanism of toxicity between cisplatin and hymenialdisine, or due to hitherto unexplained mechanisms. Therefore, study of mechanism of action of hymenialdisine would be a 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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References


**Figure 1:** (A) Viability percentage for A2780S in the presence of different concentrations of cisplatin (IC50 31.4 µM) and hymenialdisine (IC50 46.6 µM). The results are expressed as mean ± SE of three independent experiments. (B) Viability percentage for A2780CP in the presence of different concentrations of cisplatin (IC50 76.9 µM) and hymenialdisine (IC50 not reached upto 300 µM). The results are presented as mean ± SE of three independent experiments for A2780S (p<0.0001) and A2780CP (p<0.0005).