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Listeriolysin O combines with Cisplatin and Carfilzomib to promote apoptosis in ovarian cancer by modulating Bcl-2 and Bax expression

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Abstract

Background and Aims: Ovarian cancer treatment is often hindered by cisplatin resistance, frequently mediated by dysregulated apoptosis. This study investigates a novel combination therapy to overcome this resistance by targeting key apoptotic regulators. To evaluate the effects of Listeriolysin O (LLO), alone and in combination with Cis and CFZ, on the mRNA expression of the anti-apoptotic gene Bcl-2 and the pro-apoptotic gene *Bax* in cisplatin-sensitive (A2780S) and cisplatin-resistant (A2780CisR) ovarian cancer cell lines.

Methods: A2780S and A2780CisR cells were treated for 72 hours with LLO, Cis, CFZ, and their combinations (Cis/LLO, CFZ/LLO, Cis/CFZ/LLO). The relative mRNA expression levels of Bcl-2 and *Bax* were quantified using quantitative real-time PCR (qRT-PCR), with normalization to the *GAPDH* housekeeping gene.

Results: LLO monotherapy had a minimal impact on gene expression. In stark contrast, all combination regimens induced significant alterations in the Bcl-2/Bax axis. The triple-combination (Cis/CFZ/LLO) was the most potent, driving Bcl-2 expression down to 10% and 18% of control levels in A2780S and A2780CisR cells, respectively ($P<0.01$), while concurrently upregulating *Bax* expression nearly three-fold (2.94 in A2780S, 2.86 in A2780CisR; $P<0.01$). The two-drug combinations of Cis/LLO and CFZ/LLO also significantly suppressed Bcl-2 and elevated *Bax* compared to control and LLO-only groups ($P<0.05$). The overall treatment effect was statistically significant for both genes in both cell lines ($p < 0.05$).

Conclusion: The combination of LLO with cisplatin and carfilzomib promotes a profound pro-apoptotic gene expression profile in ovarian cancer cells by drastically downregulating Bcl-2 and upregulating *Bax*. This multi-mechanistic strategy, which concurrently targets DNA integrity, proteostasis, and plasma membrane integrity, effectively overcomes cisplatin resistance and represents a highly promising therapeutic approach for chemoresistant ovarian carcinoma.

Keywords: Listeriolysin O (LLO), Bcl-2, Bax, Cisplatin, Ovarian cancer.

Introduction

Ovarian cancer remains the most lethal malignancy of the female reproductive system, characterized by a frequently asymptomatic progression that often leads to late-stage diagnosis and a dismal prognosis.[1] Despite initial responsiveness to cytoreductive surgery and platinum-based chemotherapy, the five-year survival rate for advanced-stage disease remains stubbornly low, primarily due to the almost inevitable development of chemoresistance.[2] Cisplatin, a cornerstone of ovarian cancer treatment, exerts its cytotoxic effects by forming intra- and inter-strand DNA crosslinks, ultimately triggering apoptotic cell death. However, the efficacy of cisplatin is profoundly limited by both intrinsic and acquired resistance mechanisms, which represent a central, unresolved challenge in clinical oncology.[3]

The molecular underpinnings of cisplatin resistance are multifaceted and complex. Well-established mechanisms include reduced intracellular drug accumulation through altered uptake and efflux transporters, enhanced DNA repair capacity, and inactivation of cisplatin via increased glutathione conjugation.[4] More recently, the critical role of evading programmed cell death, or apoptosis, has been recognized as a paramount factor. The B-cell lymphoma 2 (Bcl-2) family of proteins serves as a critical gatekeeper of the mitochondrial apoptotic pathway, with its delicate balance determining cellular fate.[5] This family is comprised of anti-apoptotic members, such as Bcl-2 and Bcl-xL, and pro-apoptotic effectors, such as Bax and Bak. A shift in this equilibrium towards the anti-apoptotic proteins is a hallmark of many cancers, including ovarian carcinoma, and is strongly implicated in chemoresistance.[6,7] For instance, Beale et al.,[6] demonstrated that elevated Bcl-2 protein expression correlated with poor response to platinum-based therapy in ovarian cancer patients. At the cellular level, studies in cisplatin-resistant A2780 (A2780/DDP) models have consistently shown that an increased Bcl-2/Bax ratio protects cells from apoptosis, and that strategies to reverse this ratio can resensitize cells to cisplatin.[8,9] This body of evidence firmly establishes the dysregulation of the Bcl-2/Bax axis as a linchpin of the chemoresistant phenotype.

Consequently, a major therapeutic strategy has focused on developing agents that can modulate this apoptotic threshold. The proteasome inhibitor carfilzomib (CFZ), a second-generation epoxomicin derivative, has emerged as a promising candidate. Approved for the treatment of multiple myeloma, CFZ irreversibly binds the chymotrypsin-like site of the proteasome, leading to the accumulation of misfolded proteins and proteotoxic stress, which can ultimately induce apoptosis.[10] Preclinical studies in ovarian cancer have demonstrated that CFZ not only possesses single-agent cytotoxicity but also can synergize with cisplatin to overcome resistance. Zarei et al.,[11] provided foundational evidence that the combination of CFZ and cisplatin mediates synergistic cell death in both cisplatin-sensitive (A2780S) and cisplatin-resistant (A2780CisR) ovarian cancer cells, with combination indices (CI) significantly below 0.9. Furthermore, this synergy was associated with a robust downregulation of drug efflux transporters MRP1 and BCRP. Subsequent work by the same group indicated that CFZ modulates the expression of key apoptotic regulators, decreasing Bcl-2 and increasing Bax mRNA levels, thereby promoting a pro-apoptotic cellular environment.[12] These findings position CFZ as a potent sensitizer that targets the core apoptotic machinery to re-establish cisplatin sensitivity.

In parallel, novel therapeutic approaches are exploring unconventional agents to disrupt cancer cell integrity. Among these, bacterial pore-forming toxins have garnered interest for their ability to directly compromise the plasma membrane of eukaryotic cells. Listeriolysin O (LLO), a key virulence factor produced by *Listeria monocytogenes*, is a cholesterol-dependent cytolysin that forms pores in host cell membranes. While its primary role is in facilitating bacterial escape from the phagosome, its membrane-disrupting properties have been investigated for targeted drug delivery and direct oncolytic effects. The transient pore formation by LLO can induce calcium influx and other intracellular stress signals that may prime cells for apoptosis or enhance the intracellular accumulation of co-administered chemotherapeutic agents. Although the application of LLO in ovarian cancer is novel, the principle of using membrane-disrupting agents to chemosensitize cancer cells is supported by other studies. For example, the melittin component of bee venom has been shown to exhibit cooperative effects with cisplatin in ovarian cancer cells through metabolomic reprogramming.[13] Similarly, other natural compounds like oleuropein[14] and oridonin [15] have been demonstrated to reverse cisplatin resistance by targeting apoptotic pathway regulators, underscoring the viability of this approach.

The scientific premise for combining LLO with cisplatin and carfilzomib is therefore built on a multi-mechanistic foundation. Cisplatin causes DNA damage, initiating the apoptotic cascade. Carfilzomib inhibits the proteasome, preventing the degradation of pro-apoptotic factors and inhibiting anti-apoptotic pathways like NF-κB, which has also been implicated in CFZ's mechanism.[16] LLO, by contrast, acts at the most upstream level, directly perturbing the plasma membrane. This primary insult could potentially lower the overall cellular threshold for

apoptosis by inducing endoplasmic reticulum stress, dysregulating calcium homeostasis, and facilitating the intracellular penetration of both cisplatin and CFZ.[17] This triple-combination strategy represents a concerted attack on the cancer cell from multiple fronts: genomic integrity (cisplatin), protein homeostasis (carfilzomib), and cellular membrane integrity (LLO). We hypothesize that this multi-targeted assault will be particularly effective in forcing resistant cells, which have evolved mechanisms to survive single-agent insults, into an inescapable apoptotic pathway, primarily through the potent modulation of the critical Bcl-2/Bax rheostat.

Objectives

Therefore, this study aims to investigate the effects of LLO, both alone and in combination with cisplatin and carfilzomib, on the expression of the key apoptotic regulators Bcl-2 and Bax in cisplatin-sensitive and cisplatin-resistant ovarian cancer cell lines. We seek to determine whether LLO can potentiate the pro-apoptotic effects of established chemotherapeutics, thereby offering a novel and potent strategy to abrogate cisplatin resistance in ovarian cancer.

Methods

Cell lines and culture conditions

The human ovarian carcinoma cell lines A2780S (cisplatin-sensitive; NCBI Code: C461) and A2780CisR (cisplatin-resistant; NCBI Code: C454) were procured from the Pasteur Institute of Iran (Tehran, Iran). The A2780CisR cell line is a well-characterized, isogenic resistant variant generated through the continuous exposure of the parental A2780S line to increasing concentrations of cisplatin, as previously described [11, 12]. To maintain the resistant phenotype, A2780CisR cells were cultured in the presence of 1 µM cisplatin for a minimum of two to three passages prior to all experiments. Both cell lines were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Thermofisher, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, USA), 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were cultured in a humidified incubator at 37°C with 5% CO_2. The culture medium was refreshed every 2–3 days, and cells were passaged at approximately 80% confluency using a standard trypsin-EDTA protocol. All experiments were performed with cells in the logarithmic growth phase.

Reagents and drug preparation

Cisplatin (Cis) and CFZ were purchased from Sigma-Aldrich (St. Louis, MO, USA) and AdooQ BioScience (CA, USA), respectively. Recombinant LLO was prepared in ready-to-use and freeze-dried forms by the Biotechnology Department of the Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. A 50 µl of LLO toxin was added to 50-µl aliquots of the two cell lines, and the viability of the cells was analyzed after 5-min incubation at 37°C.[18] Each experiment was performed in triplicate. IC50 of the effects of toxin on the two cell lines was calculated by sigmoid regression of viability data against the toxin concentrations.

A stock solution of CFZ (200 µM) was prepared in dimethyl sulfoxide (DMSO) and stored at -20°C. Cisplatin was freshly solubilized in phosphate-buffered saline (PBS) prior to each experiment. LLO was reconstituted in *PBS* according to the manufacturer's instructions. All subsequent working concentrations were prepared by serial dilution in complete RPMI 1640 culture medium immediately before use, ensuring the final concentration of DMSO did not exceed 1% (v/v). Control groups were treated with a vehicle control of culture medium containing an equivalent concentration of DMSO.

Experimental design and treatment protocol

For gene expression analysis, cells were seeded in 6-well plates at a density of 2.5 x 10^5 cells per well and allowed to adhere for 24 hours. The concentrations for Cis and CFZ were selected based on the predetermined IC_{50} values for a 72-hour exposure from our previous work.[11,16] The concentration of LLO was determined from preliminary cytotoxicity (MTT) assays to be a sub-cytotoxic, biologically active dose. Subsequently, they were treated according to the following experimental groups for a period of 72 hours:

- **Control (Con):** Vehicle control (1% DMSO in complete medium).
- **LLO:** Treated with LLO alone (For the A2780S cell line, the IC50 value was 0.0057 µg/ml, and for the

A2780Ris cell line, the IC50 value was 0.0175 µg/ml at 72 hours).

- **Cis/LLO:** Treated with a combination of cisplatin (For the A2780S cell line, the IC50 value was 10.7 µg/ml, and for the A2780Ris cell line, the IC50 value was 20.55 µg/ml at 72 hours) and LLO.
- **CFZ/LLO:** Treated with a combination of carfilzomib (For the A2780S cell line, the IC50 value was 0.006 µg/ml, and for the A2780Ris cell line, the IC50 value was 0.218 µg/ml at 72 hours) and LLO.
- **Cis/CFZ/LLO:** Treated with the triple combination of cisplatin, carfilzomib, and LLO.

All treatments were performed in triplicate wells and repeated in at least three independent biological replicates.

RNA extraction and cDNA synthesis

Following the 72-hour treatment period, total RNA was isolated from cells using the RiboEx total RNA extraction kit (GeneAll, Korea), in strict accordance with the manufacturer's protocol. Genomic DNA contamination was removed using the provided DNase I. The concentration and purity (A260/A280 ratio of 1.8-2.0) of the extracted RNA were quantified using a NanoDrop spectrophotometer (Biotek Instruments, USA), and RNA integrity was confirmed via 1% agarose gel electrophoresis. One microgram of total RNA from each sample was reverse-transcribed into complementary DNA (cDNA) using a cDNA synthesis kit (Sinaclon, Tehran, Iran) with a blend of Oligo dT and Random Hexamer primers.

Quantitative Real-Time PCR (qRT-PCR)

The mRNA expression levels of the target genes, Bcl-2 and *Bax*, were quantified by qRT-PCR using a Rotor-Gene Q real-time PCR system (Qiagen, Germany) and the SYBR Green master mix (Yekta Tajhiz Azma, Iran). The primer sequences [11, 16] used were as follows:

Bcl-2: Forward 5′-CTCGTCCAAGAATGCAAGC-3′, Reverse 5′-CATCACTATCTCCGGTTATCG-3′ (Amplicon: 178 bp).

***Bax*:** Forward 5′-CCCGAGAGGTCTTTTTCCGAG-3′, Reverse 5′-CCAGCCCATGATGGTTCTGAT-3′ (Amplicon: 155 bp).

***GAPDH* (reference gene):** Forward 5′-CTCATTTCCTGGTATGACAACGA-3′, Reverse 5′-TCTTCCTCTTGTCCTCTTGCTG-3′ (Amplicon:123bp).

The PCR amplification protocol consisted of an initial denaturation at 95°C for 3 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds. A melt curve analysis was performed at the end of each run to confirm the specificity of amplification and the absence of primer-dimers. All reactions were performed in duplicate. The relative expression of the target genes was calculated using the $2^{-\Delta\Delta Ct}$ method, with normalization to the *GAPDH* housekeeping gene, which demonstrated stable expression across treatment groups.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) from at least three independent experiments. Statistical analyses were performed using SPSS software (version 22.0, SPSS Inc., USA) and GraphPad Prism 6.0 (GraphPad Software, USA). The normality of data distribution was assessed using the Shapiro-Wilk test. For multiple comparisons, one-way analysis of variance (ANOVA) was applied, followed by Tukey's post-hoc test for detailed group-wise comparisons. A p-value of less than 0.05 was considered statistically significant.

Ethical statement

The study protocol was reviewed and approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Approval Code: IR.SSU.MEDICINE.REC.1396.53), confirming that all procedures adhered to relevant ethical guidelines for laboratory research.

Results

We evaluated the ability of LLO to alter the expression of key apoptotic regulators, both alone and in combination with Cis and CFZ, in cisplatin-sensitive (A2780S) and cisplatin-resistant (A2780CisR) ovarian cancer cells.

Combination therapies cooperatively suppress anti-apoptotic Bcl-2

Treatment with LLO alone did not significantly alter Bcl-2 mRNA levels. In contrast, all combination regimens produced a profound and statistically significant downregulation of Bcl-2 in both cell lines ($p<0.037$ for overall treatment effect) [Table 1]. The triple-combination therapy (Cis/CFZ/LLO) was the most potent,

demonstrating the strongest suppression of Bcl-2 (P<0.01 versus control). The two-drug combinations of Cis/LLO and CFZ/LLO also significantly suppressed Bcl-2 expression compared to both the control and LLO-only groups (P < 0.01 and P < 0.05, respectively). A significant difference in the efficacy of the Cis/LLO combination was observed between the two cell lines, with the resistant A2780CisR cells maintaining higher Bcl-2 levels compared to the sensitive A2780S cells (p < 0.05) [Figure-1].

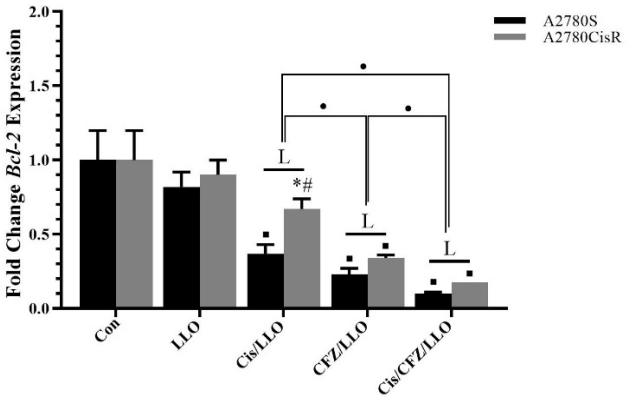

Figure 1. Relative Bcl-2 mRNA expression following treatment. Treatments, particularly the combinations, significantly suppressed Bcl-2 levels in both cell lines. The Cis/LLO combination showed a significant inter-cell-line difference (# P< 0.05). ● P< 0.05 comparison between combined groups. Data are mean ± SD; (* P< 0.05, ▪ P< 0.01 vs. control; L P< 0.05 vs. LLO group).

Pro-apoptotic Bax is upregulated by LLO-containing combinations

The effects on the pro-apoptotic gene *Bax* mirrored those on Bcl-2. LLO monotherapy had a minimal effect, whereas its combination with Cis and CFZ induced a significant upregulation of *Bax* (p<0.043 for overall treatment effect) [Table 1]. The CFZ/LLO and the triple-combination Cis/CFZ/LLO treatments were the most effective, both resulting in a marked induction of *Bax* mRNA (P<0.01 versus control and P<0.05 versus LLO alone). The Cis/LLO combination also significantly increased *Bax* expression compared to the control (p<0.05) [Figure-2].

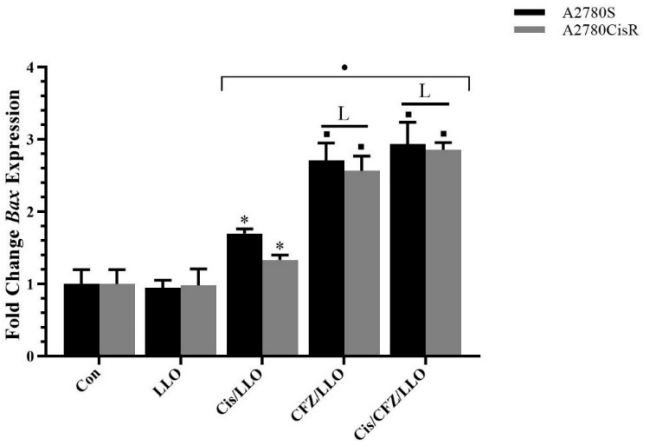

Figure 2. Relative Bax mRNA expression following treatment. Combination treatments, especially CFZ/LLO and Cis/CFZ/LLO, significantly induced *Bax* expression. ● P< 0.05 comparison between combined groups. Data are mean ± SD; (* P< 0.05, ▪ P< 0.01 vs. control; L P< 0.05 vs. LLO group

Discussion

The development of cisplatin resistance remains a formidable barrier to the successful treatment of ovarian cancer, necessitating the exploration of novel therapeutic strategies that can resensitize tumor cells to conventional chemotherapy. In this study, we investigated the potential of the pore-forming toxin LLO to modulate the apoptotic machinery and enhance the efficacy of Cis and the proteasome inhibitor CFZ in both cisplatin-sensitive (A2780S) and cisplatin-resistant (A2780CisR) ovarian cancer cell lines. Our principal finding is that LLO acts as a powerful sensitizing agent, acting in concert with Cis and CFZ to induce a profound shift in the expression of key Bcl-2 family proteins, thereby promoting a pro-apoptotic cellular state even in chemoresistant cells. This multi-mechanistic approach, targeting genomic integrity, proteostasis, and plasma membrane integrity simultaneously, represents a promising strategy to overcome the complex, multi-factorial nature of cisplatin resistance.

Table 1. Effects of LLO and its combinations on the relative mRNA expression of Bcl-2 and Bax in A2780S and A2780CisR cell lines.

Gene	Cell Line	Con	LLO	Cis/LLO	CFZ/LLO	Cis/CFZ/LLO	P value
Bcl-2	A2780S	1.00 ± 0.20	0.82 ± 0.01	0.37 ± 0.06$^{▪L}$	0.23 ± 0.04$^{▪L}$	0.10 ± 0.02$^{▪L}$	0.026
	A2780CisR	1.00 ± 0.20	0.90 ± 0.03	0.67 ± 0.07$^{*\#L}$	0.34 ± 0.02$^{▪L}$	0.18 ± 0.00$^{▪L}$	0.037
Bax	A2780S	1.00 ± 0.20	0.95 ± 0.23	1.70 ± 0.06*	2.71 ± 0.24$^{▪L}$	2.94 ± 0.30$^{▪L}$	0.036
	A2780CisR	1.00 ± 0.20	0.98 ± 0.03	1.33 ± 0.07*	2.57 ± 0.20$^{▪L}$	2.86 ± 0.10$^{▪L}$	0.043

Data presented as mean ± SD. * P< 0.05 and ▪ P< 0.01 compared to the control group; # P< 0.05 compared to the other cell line; L P< 0.05 compared to the LLO group.

The central role of the Bcl-2 family in regulating the mitochondrial apoptotic pathway and its dysregulation in chemoresistance is well-documented.[6,7] Our data confirm this paradigm, demonstrating that successful therapeutic intervention is associated with a significant downregulation of the anti-apoptotic protein Bcl-2 and a concurrent upregulation of the pro-apoptotic effector Bax. While LLO monotherapy had only a modest effect, its combination with chemotherapeutic agents was strikingly potent. The triple-combination therapy (Cis/CFZ/LLO) emerged as the most effective regimen, driving Bcl-2 expression down to a mere 10% and 18% of control levels in A2780S and A2780CisR cells, respectively. This was accompanied by a near three-fold induction of Bax. This dramatic alteration of the Bcl-2/Bax ratio is critically important, as it favors the formation of Bax oligomers in the mitochondrial membrane, leading to cytochrome c release and the irreversible initiation of the caspase cascade.[5] The efficacy of this combination in the resistant A2780CisR line is particularly noteworthy. The persistence of a higher Bcl-2 level in A2780CisR cells treated with Cis/LLO compared to A2780S cells highlights the robustness of the resistant phenotype, a phenomenon also observed by others.[9] However, the fact that the triple-combination could overcome this difference and induce such a strong pro-apoptotic signal underscores its superior capacity to bypass resistance mechanisms.

The cooperative effect we observed can be attributed to the complementary mechanisms of action of the three agents. Cisplatin's primary role is to inflict DNA damage, which typically signals through p53 to upregulate pro-apoptotic Bax and downregulate anti-apoptotic Bcl-2.[19] However, in resistant cells, this pathway is often compromised. This is where CFZ appears to play a crucial role. As an irreversible proteasome inhibitor, CFZ prevents the degradation of numerous regulatory proteins, including pro-apoptotic factors and inhibitors of NF-κB, a transcription factor known to promote the expression of anti-apoptotic genes like Bcl-2.[11,16] Our findings align with previous work showing that CFZ can downregulate Bcl-2 and upregulate Bax,[12] and that it synergizes with cisplatin.[11] By inhibiting the proteasome, CFZ likely stabilizes proteins that promote apoptosis and blocks pro-survival signals, thereby lowering the threshold for cell death initiation.

The novel component of our strategy is the incorporation of LLO. We propose that LLO acts as a critical "priming" agent that exacerbates the cellular stress induced by Cis and CFZ. The primary action of LLO is the formation of transient pores in the plasma membrane. This membrane disruption can have several downstream consequences that potentiate apoptosis. Firstly, it can lead to a dysregulation of ion homeostasis, particularly calcium influx, which is a known potent inducer of endoplasmic reticulum (ER) stress and mitochondrial permeability.[20] ER stress, in turn, can activate the unfolded protein response and engage apoptotic pathways independently of DNA damage, providing a parallel route to cell death that may be intact in cisplatin-resistant cells.[21] Secondly, membrane poration could facilitate the increased intracellular uptake of both cisplatin and carfilzomib. While we did not directly measure intracellular drug concentrations in this study, enhanced drug accumulation is a well-established mechanism for reversing resistance, as one of the key defenses of resistant cells is to limit the intracellular buildup of chemotherapeutics.[22] By physically breaching this barrier, LLO may directly counteract this resistance mechanism. This concept is supported by studies using other membrane-active agents, such as melittin, which have shown synergistic effects with cisplatin.[13]

The convergence of these three distinct insults -genotoxic stress (Cis), proteotoxic stress (CFZ), and membrane/ionic stress (LLO)- likely creates an insurmountable level of cellular damage. Resistant cancer cells often develop adaptive mechanisms to cope with a single type of stress, but simultaneously attacking from multiple directions overwhelms these compensatory networks. This is elegantly reflected in our gene expression data, where the triple-combination produced the most dramatic and statistically significant shift in the Bcl-2/Bax ratio. The resulting pro-apoptotic environment forces the cell down a death pathway that it can no longer evade. This multi-targeted approach is consistent with the emerging consensus that overcoming robust chemoresistance requires combination therapies that address its

heterogeneous mechanisms.[23, 24]

Our findings must be considered in the context of the study's limitations. Firstly, these results are derived from in vitro models. The tumor microenvironment in vivo, with its complex stromal interactions, hypoxic regions, and immune cell involvement, could modulate the efficacy and specificity of this combination therapy. For instance, hypoxia has been shown to alter the effects of chemosensitizers like resveratrol.[25] Future studies in patient-derived xenograft models will be essential to validate these findings and assess potential toxicity. Secondly, our data are based on mRNA expression. While qRT-PCR is a robust and quantitative method, confirming these changes at the protein level via Western blotting and demonstrating functional apoptosis through assays like caspase-3/7 activation would provide a more comprehensive picture of the apoptotic cascade. Furthermore, investigating the precise signaling pathways upstream of Bcl-2/Bax modulation, such as the involvement of p53, AKT, or ERK, would yield deeper mechanistic insights. For example, the AKT pathway is a known regulator of Bcl-2 family proteins in cisplatin-resistant ovarian cancer,[9] and its status in the context of LLO treatment warrants investigation.

Despite these limitations, the implications of our work are significant. The ability of LLO to dramatically enhance the pro-apoptotic effects of established chemotherapeutics opens a new avenue for adjuvant therapy in cisplatin-resistant ovarian cancer. It suggests that agents targeting physical cell membrane integrity, a vulnerability less prone to the molecular adaptations that cause drug resistance, can be powerful tools in the oncologist's arsenal. Future research should focus on optimizing the delivery of LLO to ensure tumor-specific targeting, thus minimizing potential off-target effects on healthy tissues. Exploring the combination of LLO with other targeted agents, such as PARP inhibitors, could also be a fruitful direction, given the interplay between DNA damage response and apoptotic signaling.

Conclusions

This study demonstrates that the pore-forming toxin LLO acts in combination with cisplatin and carfilzomib to potently induce a pro-apoptotic gene expression profile in ovarian cancer cells, effectively overcoming cisplatin resistance. The triple-combination therapy forces a critical rebalancing of the Bcl-2/Bax axis, creating a cellular environment that is permissive for apoptosis. By concurrently attacking the cancer cell through genomic, proteostatic, and membrane-based mechanisms, this strategy presents a compelling and multi-faceted approach to dismantling the robust defenses of chemoresistant ovarian carcinoma, offering a promising foundation for the development of new therapeutic regimens for this devastating disease.

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Competing interests

The authors declare that they have no competing interests.

Abbreviations

Listeriolysin O: LLO; B-cell lymphoma 2: Bcl-2; Bcl-2-associated X protein: Bax; Bcl-2 homologous antagonist/killer: Bak; Carfilzomib: CFZ; Nuclear Factor kappa-light-chain-enhancer of activated B cells: NF-κB; Roswell Park Memorial Institute : RPMI; Fetal Bovine Serum: FBS; Dimethyl Sulfoxide: DMSO; Phosphate-Buffered Saline: PBS; Half-Maximal Inhibitory Concentration: IC_{50}; Quantitative Real-Time Polymerase Chain Reaction: qRT-PCR; Glyceraldehyde-3-Phosphate Dehydrogenase: GAPDH; Standard Deviation: SD; Analysis of Variance: ANOVA; Endoplasmic Reticulum: ER; Unfolded Protein Response: UPR; Poly (ADP-ribose) Polymerase: PARP.

Authors' contributions

All authors read and approved the final manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

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Role of the funding source

None.

Availability of data and materials

The data used in this study are available from the

corresponding author on request.

Ethics approval and consent to participate

The study protocol was reviewed and approved by the Ethics Committee of Shahid Sadoughi University of Medical Sciences, Yazd, Iran (Approval Code: IR.SSU.MEDICINE.REC.1396.53).

Consent for publication

By submitting this document, the authors declare their consent for the final accepted version of the manuscript to be considered for publication.

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