MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Exploring Synergistic Effects of Combined Chemotherapeutic and Statin Therapy in Triple-Negative Breast Cancer

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Received:26/3/2024
Accepted: 24/4/2024

Abstract: Breast cancer remains one of the most common malignancies affecting women worldwide, with triple-negative breast cancer (TNBC) representing a particularly aggressive and treatment-resistant subtype. TNBC does not express estrogen receptors, progesterone receptors, or HER2, making it ineligible for hormone or HER2-targeted therapies. Consequently, treatment options for TNBC are limited, and the prognosis is often poor. This highlights a critical need for innovative therapeutic strategies that can effectively target this challenging cancer subtype**.** This study aims to evaluates the synergistic anticancer effects of combining doxorubicin (DOX) and atorvastatin (ATO) on triple-negative breast cancer using the MDA-MB-231 cell line. The combination of DOX and ATO significantly lowered the IC50 value to 10 µM from individual IC50s of 19.9 µM (DOX) and 12.2 µM (ATO), indicating increased efficacy. Analysis using the Combination Index (CI) revealed strong synergism (CI < 1), notably at high (CI = 0.28144 at 50 µM total dose) and low concentrations (CI = 0.47037 at 3.12 µM). Additionally, the Dose Reduction Index (DRI) indicated that effective doses could be significantly reduced (DRI > 6 at Fa = 0.9), suggesting potential for lower side effects. These results advocate for the combined use of DOX and ATO in TNBC treatment and warrant further clinical validation.

keywords: Triple-negative breast cancer, combination therapy, synergy, Combination Index, Dose Reduction Index.

1.Introduction

Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer known for its resistance to conventional chemotherapeutic agents, such as doxorubicin. The resistance to doxorubicin in TNBC has been associated with various molecular mechanisms, including increased lipid metabolism. Several studies have highlighted the role of lipid metabolism in promoting doxorubicin resistance in TNBC. emphasized the significance of targeting lipid metabolism as a promising therapeutic strategy for cancer[1] They discussed the potential of hypolipidemic agents and antineoplastic agents in modulating lipid metabolism to overcome drug resistance in cancer cells. Furthermore, identified a unique morphologic-metabolic phenotype associated with chemotherapy resistance in TNBC, shedding light on novel therapeutic targets resulting from vulnerabilities in this phenotype, including the expression of perilipin-4 (PLIN4) essential for stabilizing lipid droplets in resistant cells[2]

The combination of mevalonate pathway inhibitors and chemotherapy has been investigated for its potential synergistic effects in cancer treatment. It has been shown that inhibiting the mevalonate pathway using fluvastatin or simvastatin can enhance tumor sensitivity to MEK inhibitors, resulting in tumor apoptosis [3]. Statins induce apoptosis in NSCLC cells by inhibiting the mevalonate

pathway, and when combined with erlotinib, they exhibit synergistic cytotoxic effects, particularly through the modulation of ERK/MAPK and PI3K/AKT signaling pathways[4]. Simvastatin demonstrated a synergistic interaction when combined with paclitaxel or panobinostat, on the ovarian cancer cell growth, suggesting their potential as a repurposed therapeutic strategy for ovarian cancer[5]. In a Drosophila lung cancer model and human lung adenocarcinoma A549 cells, the combination of trametinib and fluvastatin synergistically rescued lethal cancer-like phenotypes, reduced trametinib's IC50, and demonstrated statistical synergy, emphasizing their combined therapeutic potential for non-small cell lung cancer[6]. Combination therapy using ABL allosteric inhibitors, such as ABL001, and statins, like simvastatin, targets the metabolic vulnerabilities of lung cancer cells, leading to a marked increase in tumor cell apoptosis, especially in brain metastatic and gefitinib-resistant models, offering a promising therapeutic approach for patients with advanced, therapy-resistant, or metastatic non-small cell lung cancer[7].

Several studies have reported that atorvastatin exhibits anti-breast cancer activity by inhibiting cell viability, migration, and proliferation, as well as inducing apoptosis and autophagy[8–16]. The anti-migration effect of atorvastatin was found to be *via* the downregulation of matrix metalloproteinases MMP-2 and MMP-9[17]. Moreover, growing evidence has demonstrated that statins, including atorvastatin, possess anti-inflammatory and immunomodulatory effects, which contribute to their potential anticancer properties[18]. Another potential mechanism by which atorvastatin may exert its cytotoxic effect in breast cancer involves the inhibition of angiogenesis, which is a critical process in tumor growth and metastasis, by decreasing the expression of vascular endothelial growth factor (VEGF)[19,20]. The combination of atorvastatin with other anticancer agents, such as zoledronic acid has demonstrated synergistic inhibitory effects on breast cancer cell proliferation and adhesion[21]. Furthermore, atorvastatin improved the sensitivity of breast cancer cells to cisplatin through the modulation of cholesterol biosynthesis[22].

2. Materials and methods

Cell Culture

MDA-MB-231 cells, a human breast cancer cell line, were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM, Biowest, Nuaille, France) supplemented with 10% fetal bovine serum (FBS, Biowest, Nuaille, France) and 1% penicillin-streptomycin(Biowest, Nuaille, France). The cells were maintained in a humidified incubator at 37°C with 5% CO2. Prior to the assays, cells were passaged using 0.25% trypsin-EDTA solution.

MTT Assay

For the MTT assay, MDA-MB-231 cells were seeded at a density of 5 × 10^3 cells per well in 96-well plates. The plates were then incubated overnight in a 37°C, 5% CO2 incubator to allow for cell adherence and recovery.

The cytotoxic effects of doxorubicin and atorvastatin were assessed using a serial dilution technique to determine the half maximal inhibitory concentration (IC50) values. Stock solutions of doxorubicin and atorvastatin were prepared in dimethyl sulfoxide (DMSO) and further diluted in culture medium to achieve final concentrations ranging from 25 μM to 0.78 μM. After 24 hours of cell seeding, the medium was replaced with drug-containing medium at various concentrations, and the cells were incubated for 48 hours under the same culture conditions.

Post-treatment, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was added to each well in accordance with the manufacturer's instructions. The plates were then incubated for 4 hours at 37°C to allow viable cells to convert the MTT into formazan, a purple precipitate. Following incubation, the medium was carefully aspirated without disturbing the precipitated formazan. To dissolve the formazan crystals, a solution containing 10% Sodium Dodecyl Sulfate (SDS) in 0.01 M HCl was added to each well. The plates were then incubated overnight at 37°C to ensure complete dissolution of the formazan product. The absorbance of the resulting solution was measured at 570 nm using a microplate reader, with a background subtraction at 630 nm.

Combination Drug Treatment and Analysis

For combination drug treatments, MDA-MB-231 cells were exposed to a 1:1 molar ratio of doxorubicin and atorvastatin, alongside their respective single treatments, across a concentration range of 25 µM to 0.78 µM. After 48 hours of treatment, the MTT assay was performed following protocol as described above.

To assess the degree of interaction between doxorubicin and atorvastatin, the fractions affected (indicative of cell death) were calculated from the MTT assay results. These data were then inputted into CompuSyn software to compute the combination index (CI) for each drug combination at various effect levels. The CI values were interpreted as follows: CI < 1 indicates synergism, CI = 1 indicates an additive effect, and CI > 1 indicates antagonism between the drugs

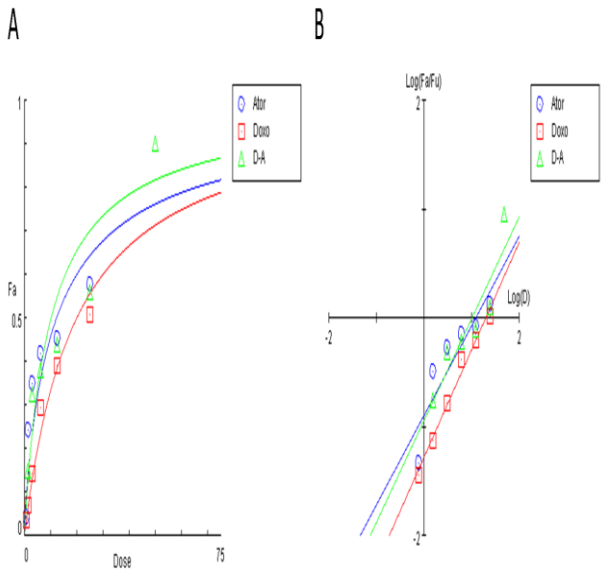

Figure- 1(A) Dose effect plot of DOX, ATO and their combination, (B) Median effect plot of DOX, ATO and their combination

3. Results and Discussion

In the present study, we assessed the anticancer effects of DOX and ATO in isolation and combined. When administered separately, DOX and ATO yielded IC50 values of 19.9 µM and 12.2 µM, respectively, aligning with findings from previous studies indicating their moderate efficacy in cancer treatment [23]. Remarkably, their combination resulted in a significantly reduced IC50 value of 10 µM (Figure 1A), echoing the enhanced therapeutic effects observed in concurrent drug regimens [24].

The dose-effect plot (Figure 1A) and median effect analysis (Figure 1B) further corroborated the increased efficacy of the drug combination, showing a pronounced slope compared to single-agent treatments. To quantify this interaction between DOX and ATO, we employed the Combination Index (CI) methodology, which has been widely recognized for distinguishing between synergistic, additive, and antagonistic drug interactions [25,26]. The calculated CI values predominantly indicated synergism (CI < 1) at various doses, particularly at the extremes of the dosing spectrum, with a shift towards additive effects (CI ≈ 1) at mid-range doses (**Table** *Error! No text of specified style in document.-**1***, Figure 2 A).

Table Error! No text of specified style in document.**-1** CI values for actual experimental points.

Total Dose	Fa	CI Value
50.0	0.9	0.28144
25.0	0.56	1.25792
12.5	0.44	1.08574
6.25	0.38	0.71973
3.12	0.326	0.47037
1.56	0.15	0.74483

Additionally, the examination of the Dose Reduction Index (DRI) offered insights into the practical benefits of this combination therapy. Notably, the DRI values suggest that lower doses of DOX and ATO could be used while maintaining efficacy, potentially reducing the adverse side effects commonly associated with these drugs[27].

Specifically, at an Fa of 0.9, the combination allowed for a significant dose reduction (Figure

(A) Combination index plot of DOX and **2** ATO. (B) Dose reduction index plot for DOX and ATO

Table-2 Figure 2 B), emphasizing the importance of DRI in evaluating the clinical utility of drug combinations.

While these results are promising, it's crucial to contextualize them within the broader spectrum of cancer therapy research. The enhanced efficacy of combining DOX and ATO offers a compelling case for reducing drug-related toxicity while maintaining, or even improving,

therapeutic outcome. However, the translation of these findings into clinical practice requires rigorous validation through clinical trials and a thorough understanding of the underlying biological mechanisms.

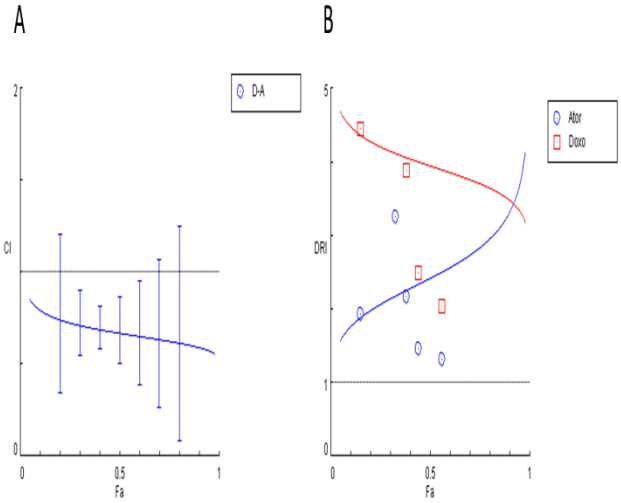

Figure 2 (A) Combination index plot of DOX and ATO. (B) Dose reduction index plot for DOX and ATO

Table-2 DRI values calculated at experimental points.

Fa	Dose Ator	Dose Doxo	DRI Ator	DRI Doxo
0.9	173.7	181.8	6.9	7.3
0.56	16.3	25.4	1.3	2.0
0.44	9.1	15.6	1.5	2.5
0.38	6.8	12.2	2.2	3.9
0.326	5.1	9.6	3.3	6.1
0.15	1.5	3.5	1.9	4.5

Conclusion and recommendations

In conclusion, our study adds to the growing body of evidence supporting the use of drug combinations over single-agent treatments in cancer therapy. The observed synergistic effects between DOX and ATO, as evidenced by lower IC50 values, favorable CI, and DRI indices, highlight the potential for more effective and less toxic treatment strategies. Further research should aim at elucidating the mechanistic basis of these interactions and validating their effectiveness and safety in clinical settings.

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