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Araştırma Makale/ Research article

Evaluation of Paclitaxel and Cisplatin Combinations in TNBC: A Strategy to Enhance Efficacy and Minimize Exposure

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ABSTRACT
Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer with limited therapeutic options due to the lack of hormone and HER2 receptors. Conventional chemotherapies such as cisplatin and paclitaxel remain important treatment options, but their efficacy is often limited by toxicity and resistance.
This study aimed to characterize the time-dependent cytotoxic and synergistic interaction profile of cisplatin and paclitaxel in TNBC cell models. Two TNBC cell lines (MDA-MB-231 and 4T1) and one non-cancerous human cell line (HEK293) were treated with increasing concentrations of cisplatin (1–25 µM) and paclitaxel (0.005–0.5 µM) for 24, 48, and 72 h. Cell viability was assessed using the MTT assay, and IC_{50} values were calculated. Drug combinations were evaluated at 24 and 48 h using the Chou–Talalay method and CompuSyn software to determine the combination index (CI). Both cisplatin and paclitaxel showed dose- and time-dependent cytotoxicity. Combination treatments demonstrated synergistic effects (CI < 1) in both TNBC cell lines. Comparable cytotoxic effects were observed at 48 h relative to 72 h single-agent exposure.These findings provide preliminary evidence of drug interaction in TNBC cell models and suggest that optimizing exposure schedules may enhance in vitro efficacy. However, further mechanistic and in vivo studies are necessary to clarify the underlying mechanisms and determine translational relevance.

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Introduction

Breast cancer remains the most frequently diagnosed cancer and the leading cause of cancer-related mortality among women worldwide [1]. Among its subtypes, triple-negative breast cancer (TNBC) accounts for approximately 10–20% of all breast cancer cases and is characterized by the absence of estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 (HER2) expression [2]. This lack of targetable receptors significantly limits therapeutic options and is associated with a more aggressive clinical course, higher recurrence rates, and poor prognosis compared to other subtypes [3].
Systemic chemotherapy remains the cornerstone of TNBC management, particularly in advanced disease. However, intrinsic and acquired resistance to chemotherapeutic agents frequently limits long-term efficacy, highlighting the importance of optimizing existing treatment regimens rather than relying solely on novel drug discovery [4], [5], [6]. In this context, combination strategies have gained attention for their potential to enhance cytotoxic efficacy and modulate treatment response dynamics [7].
Paclitaxel and cisplatin are two widely used chemotherapeutic agents with distinct mechanisms of action. Paclitaxel stabilizes microtubules and inhibits mitotic division, while cisplatin induces DNA cross-linking and triggers apoptosis [8]. Their combination has been investigated in multiple solid tumors, including breast cancer, with reports of additive or synergistic effects [9], [10], [11]. However, limited studies have comparatively evaluated the time-dependent interaction profile of this combination specifically in TNBC models, particularly in parallel human and murine systems and in comparison with non-cancerous cells to assess differential cytotoxic responses.
Therefore, the present study aimed to characterize the time-dependent cytotoxic and synergistic interaction patterns of paclitaxel and cisplatin in two TNBC cell lines (MDA-MB-231 and 4T1), alongside a non-

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cancerous control (HEK293). By focusing on early exposure intervals (24–48 h) and comparative response profiling, this study seeks to provide a refined in vitro evaluation of combination dynamics rather than to propose a novel drug pairing.

Material and Methods

Cell lines and reagents

In this study, three cell lines were used to evaluate the combinatory effect of paclitaxel and cisplatin: two cancerous cell lines and one non-cancerous cell line. MDA-MB-231 human breast cancer cells were cultured in RPMI-1640 medium, 4T1 mouse mammary carcinoma cells were grown in Dulbecco's Modified Eagle Medium (DMEM), and HEK-293 human embryonic kidney cells were maintained in Eagle's Minimum Essential Medium (EMEM). All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin-streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. Cell growth and morphology were monitored daily using an inverted microscope (Zeiss PrimoVert). The morphological effects of paclitaxel and cisplatin were also examined using a Zeiss PrimoVert inverted microscope. Cisplatin was used in its clinical intravenous formulation (25 mg/25 mL; ready-to-use solution) and diluted directly in culture medium to obtain working concentrations. Paclitaxel was similarly used in its injectable formulation (30 mg/5 mL) and diluted in culture medium to desired concentrations. No additional organic solvents were used for stock preparation. Therefore, separate solvent control groups were not required.

MTT Cell viability assay

The cytotoxic effects of paclitaxel and cisplatin were evaluated using the MTT assay. Cells were seeded at a density of 1×10^4 cells per well in 96-well plates and treated with increasing concentrations of paclitaxel (0.005, 0.01, 0.05, 0.1, 0.25, 0.5 µM) or cisplatin (1, 2.5, 5, 10, 25 µM). Following incubation, 10 µL of MTT solution was added to each well, and the plates were incubated for 4 hours at 37 °C. After incubation, the culture medium was removed and DMSO was added at equal final volume to all wells to solubilize the formazan crystals. DMSO was used only at this final step of the assay and was not present during drug treatment. Cell viability was expressed as a percentage of the untreated control group, which was defined as 100% viability. Absorbance was recorded at 570 nm with a reference wavelength of 680 nm (TECAN Infinite 200 Pro), and viability was calculated as:

$$\% \text{ Viability} = (\text{OD_treated} / \text{OD_control}) \times 100$$

IC_{50} values were calculated from dose–response curves generated using non-linear regression (variable slope model) in GraphPad Prism. The curves were constructed from the mean viability values obtained from three independent biological experiments.

Combination analysis

Drug interaction analysis was performed using the Chou–Talalay method based on the median-effect principle [12], [13]. Combination experiments were conducted using a non-constant ratio design, in which multiple concentration pairs of cisplatin (2.5–25 µM) and paclitaxel (0.005–0.05 µM) were tested across a dose matrix (16 combinations per time point).

Cell viability values obtained from three independent biological experiments were averaged and converted to fraction affected (Fa = 1 − viability fraction). The mean Fa values were entered into CompuSyn software (ComboSyn Inc., USA) to calculate combination index (CI) values at corresponding effect levels. CI < 1, CI = 1, and CI > 1 were interpreted as synergistic, additive, and antagonistic interactions, respectively.

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test with GraphPad Prism 8. All experiments were performed in three independent biological replicates (n = 3). Statistical significance was determined relative to untreated controls ($p < 0.05$).

Results

Cytotoxic Effects of Cisplatin on Cell Viability

To determine the cytotoxic effects of cisplatin, MDA-MB-231, 4T1, and HEK293 cells were treated with increasing concentrations (1, 2.5, 5, 10, and 25 µM) for 24, 48, and 72 h. A dose- and time-dependent decrease in cell viability was observed across all cell lines (Figure 1). The reduction in viability was more pronounced in cancer cell lines compared to the non-cancerous HEK293 cells.

IC_{50} values for cisplatin revealed that MDA-MB-231 cells were more sensitive to the drug after 72 h (11.49 µM), compared to 24 and 48 h (19.50 µM and 24.11 µM, respectively). Similarly, 4T1 cells showed increased sensitivity at 72 h (15.27 µM) compared to 24 and 48 h (20.46 µM and 24.23 µM, respectively).

HEK293 cells remained less sensitive overall, with the IC_{50} reaching 26.20 µM at 24 hours, decreasing to 17.65 µM at 48 hours, and slightly increasing again to 19.93 µM at 72 h (Table 1).

Cytotoxic Effects of Paclitaxel on Cell Viability

Paclitaxel treatment (0.005 to 0.5 µM) also resulted in a dose- and time-dependent inhibition of cell proliferation in all three cell lines (Figure 2). The IC_{50} values for MDA-MB-231 cells were 0.401 µM, 0.155 µM, and 0.045 µM at 24, 48, and 72 h, respectively, indicating increased sensitivity over time. A similar trend was observed for 4T1 cells, with IC_{50} values decreasing from 0.494 µM (24 h) to 0.141 µM (72 h). Notably, HEK293 cells exhibited lower IC_{50} values than the cancer lines at 72 h (0.034 µM), (Table 1).

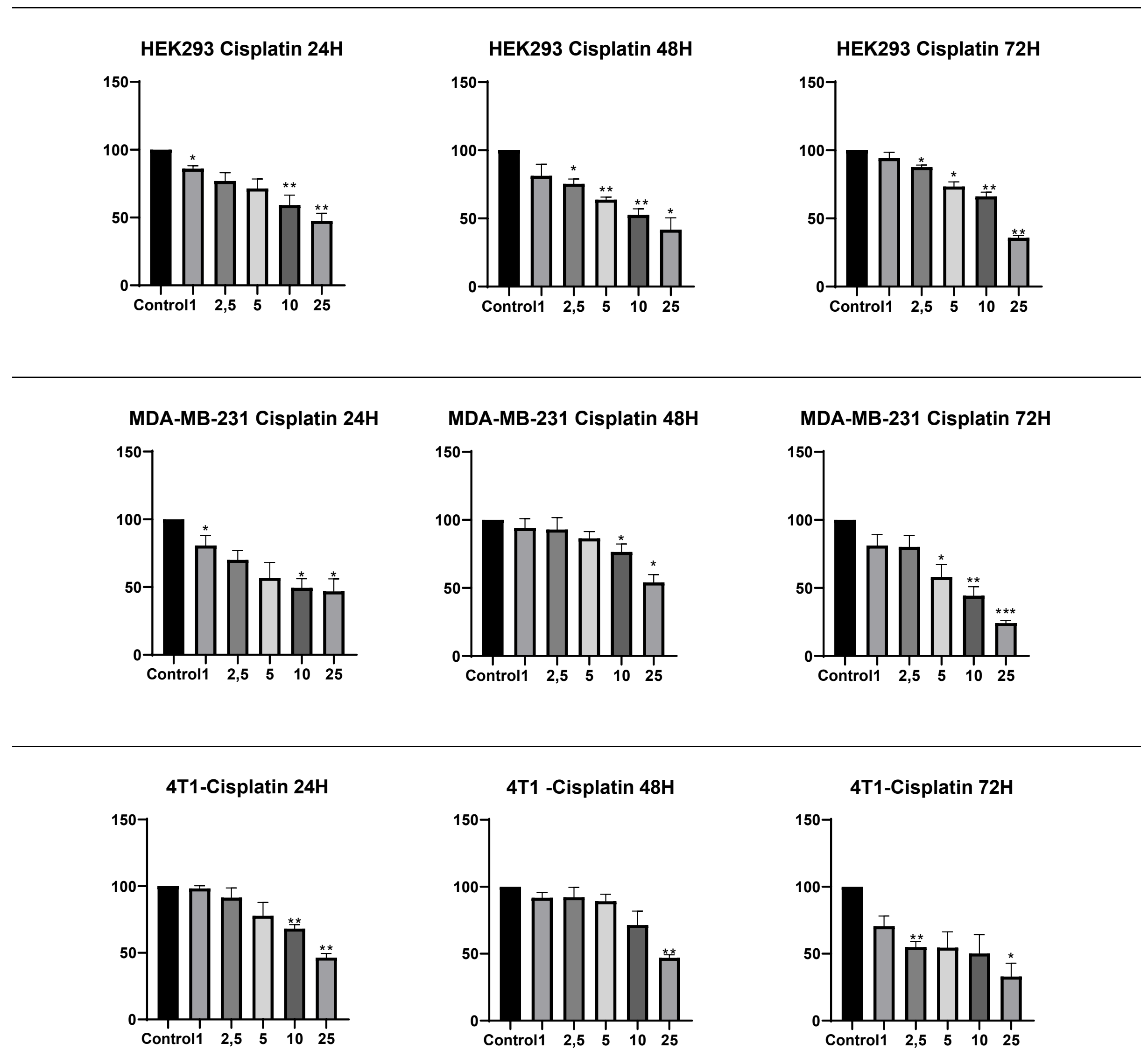


Fig 1. Cytotoxic effects of cisplatin on MDA-MB-231, 4T1, and HEK293 cells following 24, 48, and 72 h treatments. Cells were treated with increasing concentrations of cisplatin (1, 2.5, 5, 10, and 25 µM). Data represent the mean of three independent experiments (n = 3).

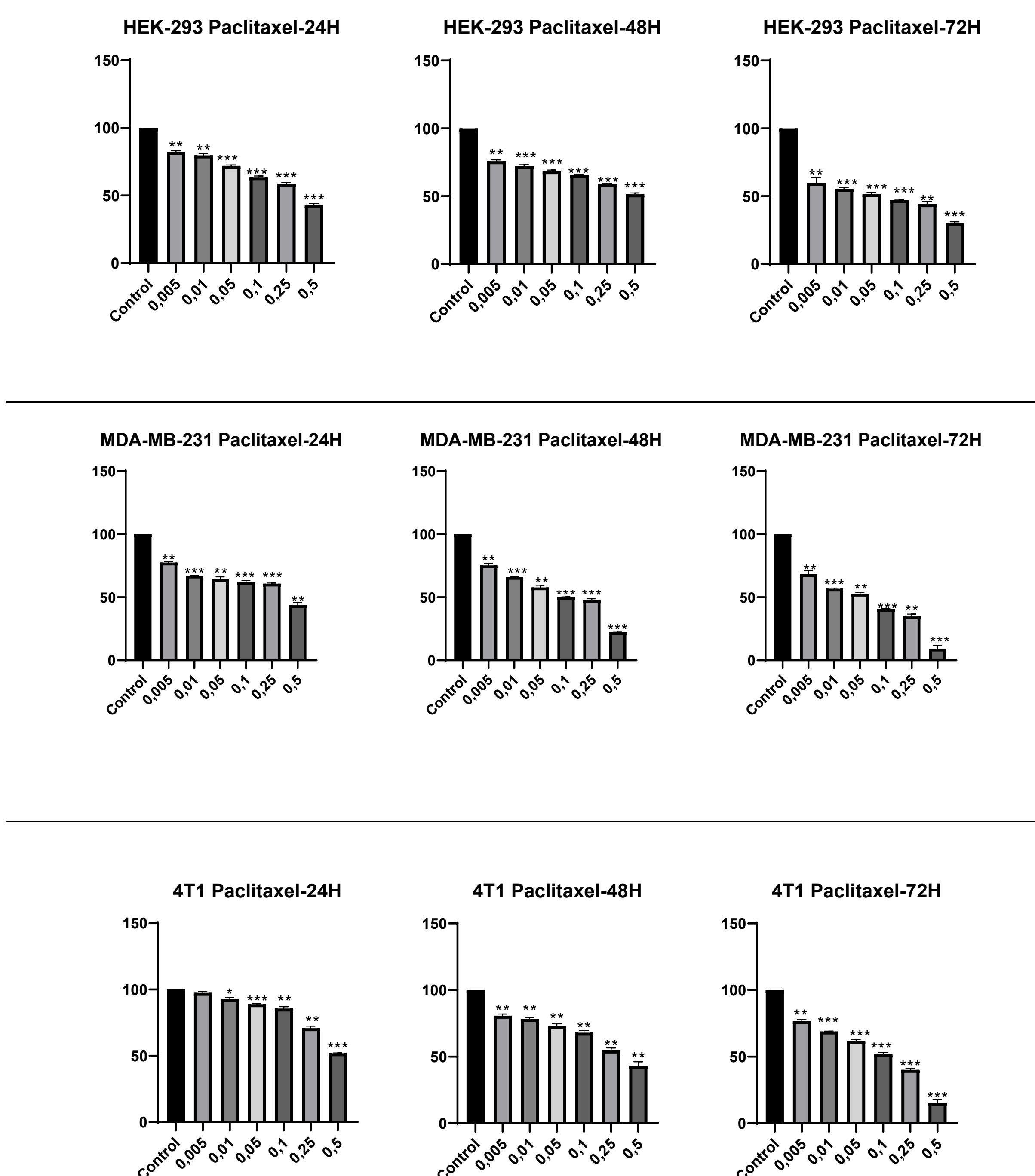


Fig 2. Cytotoxic effects of paclitaxel on MDA-MB-231, 4T1, and HEK293 cells following 24, 48, and 72 h treatments. Cells were exposed to paclitaxel at concentrations 0.005, 0.01, 0.05, 0.1, 0.25, 0.5 µM. Data represent the mean of three independent experiments (n = 3).

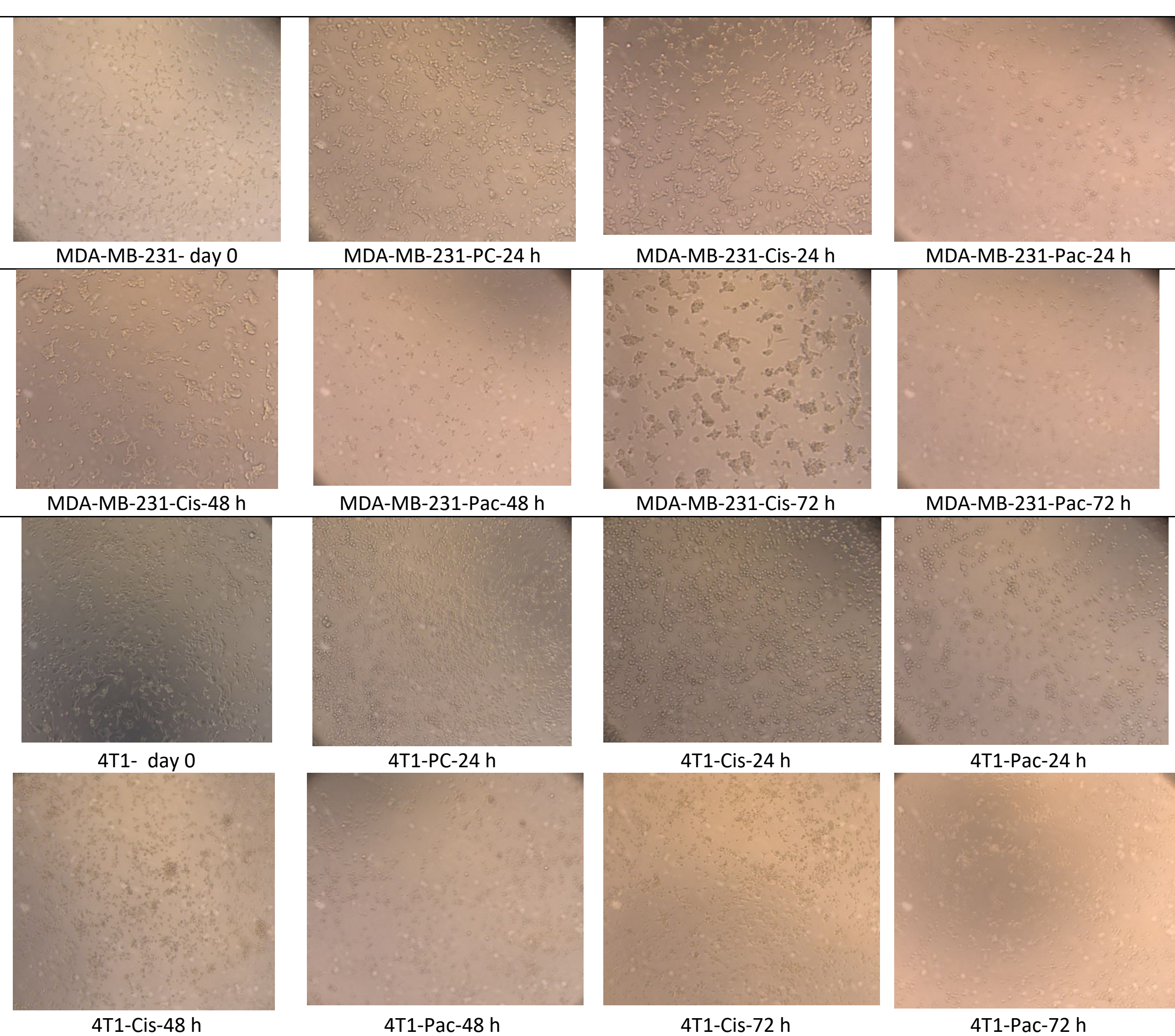
Table 1. IC_{50} values calculated from dose–response curves in MDA-MB-231, 4T1, and HEK293 cells following 24, 48, and 72 h treatments.

Cisplatin (IC_{50}, µM)		Cisplatin (IC_{50}, µM)	
HEK-293		HEK-293	
24	26.20 µM	24	0.361 µM
48	17.65 µM	48	0.509 µM
72	19.93 µM	72	0.034 µM

MDA-MB-231		MDA-MB-231	
24	19.50 µM	24h	0.401 µM
48	24.11 µM	48h	0.155 µM
72	11.49 µM	72h	0.045 µM
4T1		4T1	
24	20.46 µM	24h	0.494 µM
48	24.23 µM	48h	0.348 µM
72	15.27 µM	72h	0.141 µM

Morphological Changes Induced by Treatments

Morphological observations under the inverted microscope revealed distinct cytotoxic effects after treatment with cisplatin and paclitaxel (Figure 3). Both agents induced noticeable cellular shrinkage, detachment, and loss of adherence in a time-dependent manner in MDA-MB-231 and 4T1 cells. These changes became more prominent at 48 and 72 h, supporting the MTT findings. HEK293 cells also showed changes in morphology upon treatment, though to a lesser extent.



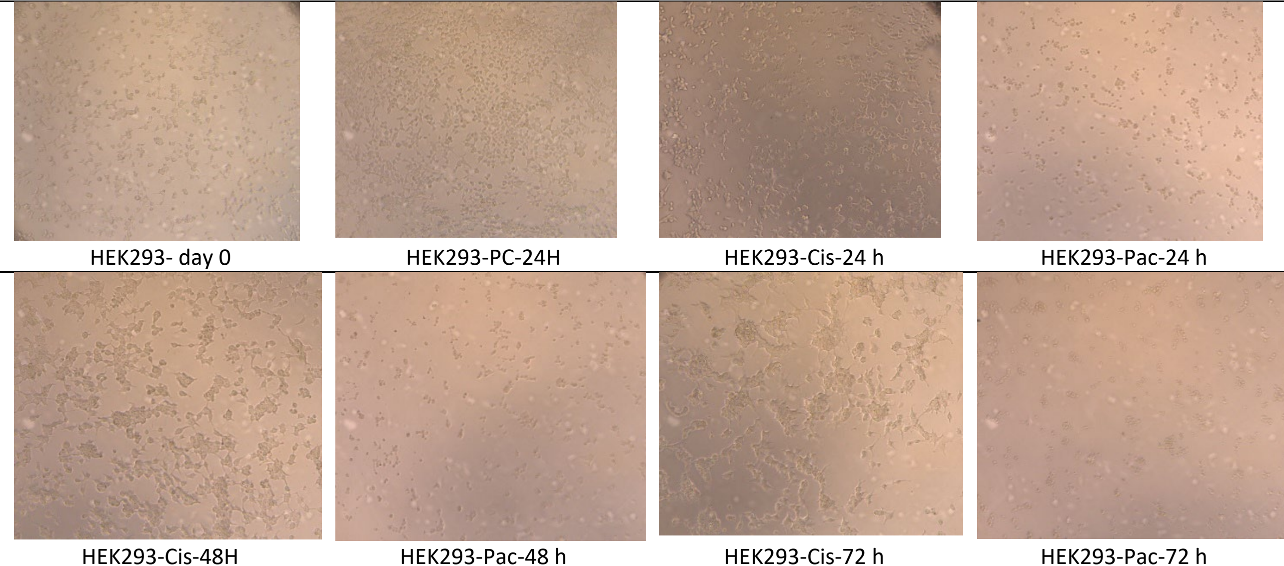


Fig 3. Microscopy images of MDA-MB-231, 4T1, and HEK293 cells treated with cisplatin and paclitaxel for 24, 48, and 72 h. Morphological changes were observed under an inverted microscope (10X) following treatment with cisplatin (25 µM) and paclitaxel (0.5 µM). Images are presented for qualitative assessment of cytotoxic effects. PC indicates Positive Control.

Combination Treatments and Synergistic Effects

To evaluate the potential synergistic effects of cisplatin and paclitaxel, cells were treated with combinations of both agents at various concentrations for 24 and 48 h. The drug interaction was quantified using the combination index (CI) method. A CI value <1 indicates synergy, and <0.5 indicates strong synergy.

As shown in Table 2, the combination of cisplatin (2.5 or 5.0 µM) with paclitaxel (0.005–0.025 µM) produced synergistic effects (CI < 0.5) in both MDA-MB-231 and 4T1 cell lines, particularly at 48 hours. For instance, the combination of 2.5 µM cisplatin with 0.025 µM paclitaxel in MDA-MB-231 cells resulted in a CI of 0.15 at 48 h. 4T1 cells showed a similar response, with CI values ranging between 0.24 and 0.28 for effective combinations. In contrast, HEK293 cells displayed only mild synergy (CI: 0.24–0.30), suggesting that the combination may selectively target cancer cells with reduced cytotoxicity toward non-malignant cells.

Fa values covered a range of effect levels across cell lines and time points. While several combinations clustered around the ED50–ED75 interval (Fa ≈ 0.4–0.7), higher-dose combinations—particularly in 4T1 cells at 48 h—also yielded lower Fa values (<0.3), indicating stronger cytotoxic responses. Synergistic CI values were observed across both moderate and higher effect levels. Graphical representation of combination cytotoxicity further confirmed the synergistic interaction of the two drugs in both cancer cell lines (Figure 4). The most effective combinations led to enhanced cell death compared to either drug alone, especially at 48 h.

Table 2. Combination index (CI) analysis of selected representative cisplatin and paclitaxel dose pairs in MDA-MB-231, 4T1, and HEK293 cells at 24 and 48 h. Fa and CI values were generated using CompuSyn software. Interpretation of CI values follows the Chou–Talalay criteria.

	Cis (µM)	Pac (µM)	24 h Fa	24 h CI	48 h Fa	48 h CI
MDA-MB-231	2.5	0.01	0.531	0.264	0.572	0.356
MDA-MB-231	2.5	0.025	0.480	0.176	0.476	0.320
MDA-MB-231	5.0	0.025	0.456	0.279	0.476	0.378
MDA-MB-231	10.0	0.01	0.518	0.905	0.407	0.221
MDA-MB-231	25.0	0.01	0.408	0.928	0.263	0.203
4T1	2.5	0.005	0.691	0.280	0.673	0.272
4T1	5.0	0.01	0.548	0.260	0.684	0.413
4T1	10.0	0.025	0.581	0.597	0.240	0.082
4T1	25.0	0.05	0.259	0.275	0.112	0.067
HEK293	2.5	0.01	0.652	0.406	0.550	0.163
HEK293	5.0	0.01	0.567	0.301	0.544	0.241

	Cis (µM)	Pac (µM)	24 h Fa	24 h CI	48 h Fa	48 h CI
HEK293	10.0	0.01	0.665	1.125	0.528	0.467

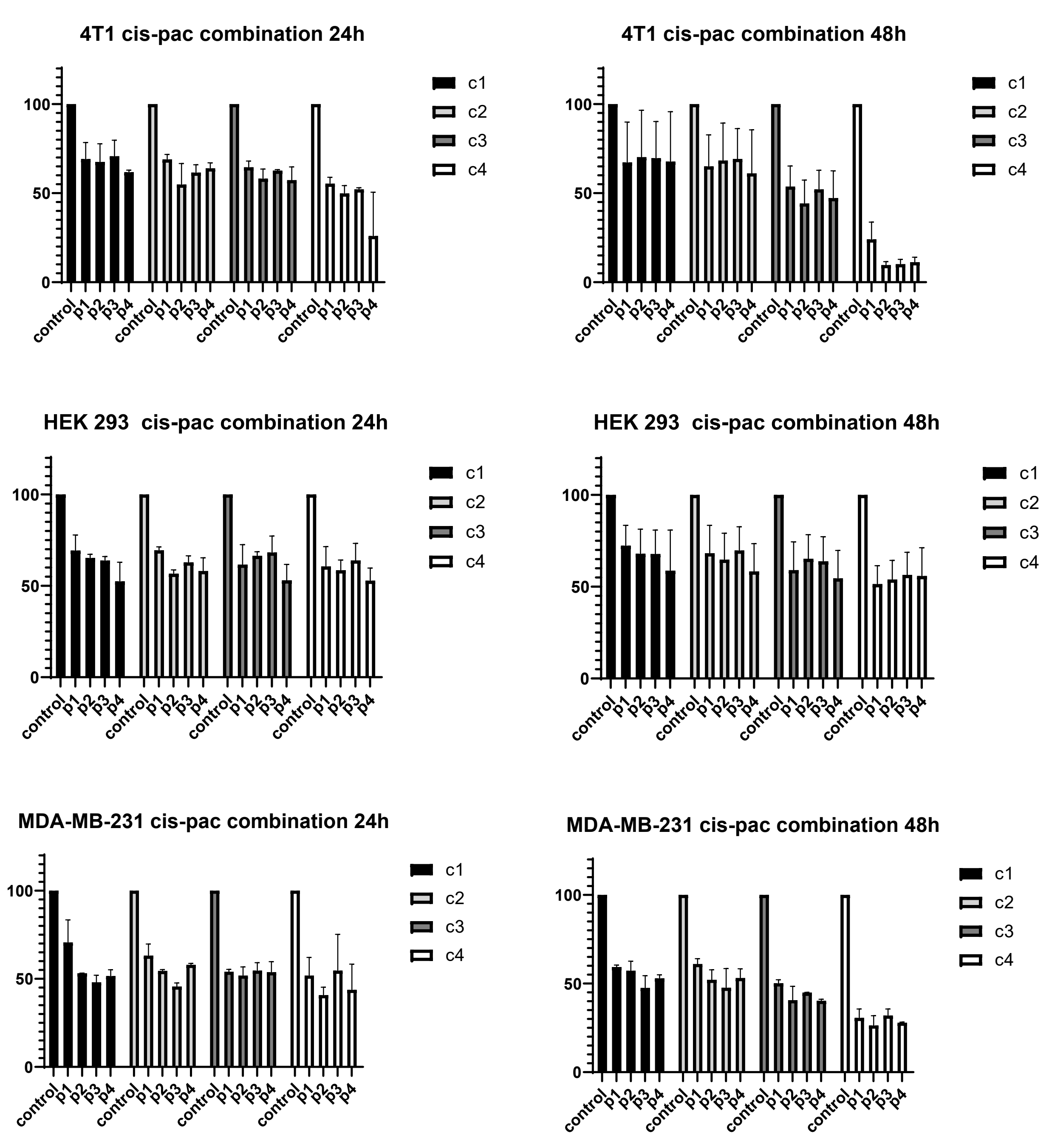


Fig 4. Effects of cisplatin and paclitaxel combinations on cell viability in MDA-MB-231, 4T1, and HEK293 cells after 24 and 48 h of treatment. Cells were treated with various combinations of cisplatin (2.5, 5, 10, 25 µM) and paclitaxel (0.005, 0.01, 0.025, 0.05 µM)

Conclusion and Discussion

In this study, the individual and combined cytotoxic effects of paclitaxel and cisplatin were investigated in two triple-negative breast cancer (TNBC) cell lines (MDA-MB-231 and 4T1) and one non-cancerous cell line

(HEK293). Although cisplatin–paclitaxel combinations have been widely investigated in other tumor types, the present study provides a time-focused interaction analysis in TNBC models, emphasizing early synergistic responses and differential cytotoxic patterns. The use of MDA-MB-231 and 4T1 cell lines in this study provides both human and murine TNBC models, which differ in their molecular signatures and metastatic behavior. This approach allows for a more comprehensive evaluation of the combination's efficacy and potential translatability to in vivo systems. HEK293 cells were included as a non-cancerous control to assess the selectivity of the drug combination. The comparatively lower sensitivity observed in HEK293 cells suggests a degree of differential cytotoxic response under in vitro conditions.

The results demonstrated that both agents exerted time- and dose-dependent cytotoxicity, with increased sensitivity observed over prolonged exposure. More importantly, the combination of cisplatin and paclitaxel yielded synergistic effects in cancer cell lines, particularly at lower concentrations and shorter incubation periods (24 and 48 h), indicating a consistent synergistic interaction pattern in TNBC cell models.

Cisplatin and paclitaxel have long been used in clinical oncology due to their distinct and complementary mechanisms of action. While cisplatin exerts its cytotoxicity through DNA crosslinking and the induction of apoptosis, paclitaxel disrupts mitotic spindle formation by stabilizing microtubules [8], [14]. Their combination studies have previously demonstrated additive or synergistic effects in a variety of solid tumors, including ovarian and lung cancers [11], [10]. The inclusion of both human (MDA-MB-231) and murine (4T1) TNBC models allowed comparison across biologically distinct systems, while HEK293 cells were used to assess differential cytotoxic responses under vitro conditions.

One of the most notable findings of this study was that synergistic interactions between paclitaxel and cisplatin were observed at early time points (24 and 48 h), without the need for extended 72 h exposure. These findings may be relevant in the context of TNBC, where treatment options are frequently constrained by resistance and cumulative toxicity. [2], [3], [20]. In this study, the combination achieved comparable or enhanced cytotoxic effects within 48 h, indicating that effective interaction between the two agents can occur without prolonged exposure [21]. Although these findings are limited to in vitro conditions, they suggest that exposure timing may be an important factor to consider in future preclinical investigations. Further in vivo studies are needed to determine whether these timing-dependent effects translate into meaningful therapeutic benefit. Several studies have demonstrated that combination approaches can enhance therapeutic efficacy compared with single-agent treatments, both in terms of exposure duration and dose optimization [15], [10], [11].

The IC_{50} analysis further supported the enhanced sensitivity of MDA-MB-231 and 4T1 cells to combination treatment. While both drugs alone were effective, the most pronounced decrease in viability was observed when used in combination, especially at 48 h. This result is clinically relevant, as shorter treatment windows are generally associated with fewer systemic side effects and improved tolerability, especially for drugs like cisplatin, which is known for dose-limiting nephrotoxicity and neurotoxicity [16]. These observations may be relevant when considering treatment scheduling strategies; however, extrapolation to systemic toxicity or clinical tolerability cannot be made based on the present in vitro data.

The combination index (CI) values were consistently below 0.5 for several concentration pairs, indicating strong synergy, particularly in cancer cell lines. In contrast, HEK293 cells showed only mild synergistic responses (CI values between 0.24 and 0.30), suggesting a degree of selectivity for malignant cells. The differential cytotoxic response observed between cancerous and non-cancerous cells may suggest a degree of selectivity under in vitro conditions; however, such implications require further validation. A more pronounced reduction in viability was detected between 24 and 48 h in the combination groups, indicating an enhanced interaction within this exposure window. As this study was limited to viability and combination index analyses, no mechanistic conclusions can be drawn. Notably, synergistic CI values were observed across different effect levels (Fa), including both moderate and stronger cytotoxic responses, supporting the consistency of the interaction pattern.

Morphological changes consistent with cytotoxic stress, including cell shrinkage and detachment, were observed, which are commonly associated with apoptotic or stress-related cell death processes in vitro [17-19]. Cellular shrinkage and detachment, more clear in TNBC cells than in HEK293. These observations are consistent with previously reported interactions between microtubule-targeting and DNA-damaging agents; however, no direct apoptotic or molecular analyses were performed in this study.

In conclusion, this study provides a focused evaluation of time-dependent synergistic cytotoxic interactions between paclitaxel and cisplatin in TNBC cell models. The findings support the presence of consistent in vitro synergy, particularly at earlier exposure times. Nonetheless, as the study was limited to viability-based analyses, further investigations incorporating molecular validation and in vivo models are necessary to clarify the biological basis and translational relevance of the observed interaction.

Abbreviations
Pac: paclitaxel, Cis: cisplatin

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Compliance with ethical standards
Conflict of interest / Çıkar çatışması
The author declare no conflict of interest.
Ethical standards
This study was conducted using commercially available cell lines and did not involve human participants or experimental animals.

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