

International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating: 5.29
IJABR 2025; 9(5): 908-912
www.biochemjournal.com
Received: 08-03-2025
Accepted: 13-04-2025

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Mechanistic insights into telmisartan-enhanced doxorubicin cytotoxicity in cancer cell lines

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DOI: <https://www.doi.org/10.33545/26174693.2025.v9.i5k.4446>

Abstract

Background: Telmisartan, an angiotensin II receptor blocker, has shown potential anticancer properties beyond its antihypertensive effects. Recent studies suggest that its combination with doxorubicin may enhance cytotoxicity in cancer cell lines through synergistic mechanisms involving apoptosis modulation and drug resistance attenuation.

Aims of the study: The aim of this study is to evaluate the synergistic cytotoxic effects of telmisartan in combination with doxorubicin on A549 lung cancer cell lines and to investigate the underlying mechanisms involved in enhancing apoptosis and reducing drug resistance.

Methodology: The study, conducted at Iraq Biotech Company in Basrah (Jan-May 2024), evaluated cytotoxic effects on A549 lung cancer cells across four treatment groups: control, Doxorubicin, Telmisartan, and their combination. Each group was exposed to six concentrations (25-800 µg/mL) for 72 hours. Standard cell culture protocols were followed using RPMI-1640 medium, supplements, and sterilized solutions. Cells were cultured, subcultured, and treated under standard conditions. Viability was assessed using Trypan blue, and cytotoxicity was measured via the MTT assay.

Results: The results showed that cell inhibition increased significantly with higher doxorubicin concentrations, with the combined treatment of doxorubicin and telmisartan producing much greater inhibition than either drug alone. The combination reached 86.78% inhibition at 800 mg/ml, showing strong synergy, especially at lower doses where increases exceeded 400%. The IC₅₀ for the combination (72.21 µg/ml) was much lower than for doxorubicin or telmisartan alone, confirming enhanced efficacy. The combination also allowed dose reductions (DRI > 8), improving therapeutic potential and reducing side effects. Overall, combined therapy significantly boosted cytotoxicity and stability compared to single treatments.

Conclusions: The combination of Doxorubicin and Telmisartan enhances cytotoxicity against A549 cells due to a synergistic effect. This is supported by reduced IC₅₀ and increased caspase-3 activity, suggesting enhanced apoptosis and potential for dose reduction and lower toxicity.

Keywords: Doxorubicin, telmisartan, A549 cell line, cytotoxicity, synergistic effect

Introduction

Cancer remains one of the most formidable global health challenges, accounting for millions of deaths annually despite significant advancements in diagnostic and therapeutic strategies. Chemotherapy, particularly with anthracyclines such as doxorubicin, has long served as a cornerstone in the treatment of a wide range of malignancies, including breast, lung, and hematological cancers [1, 2]. Doxorubicin works by damaging DNA and inhibiting topoisomerase II, causing apoptosis of rapidly multiplying cancer cells. Nonetheless, its therapeutic index is generally restricted by dose-dependent toxicities, predominantly cardiotoxicity and the induction of multidrug resistance (MDR). Such limitations emphasises significant requirements for adjuvant therapies capable of enhancing the antitumor effect of doxorubicin, and reducing the side effects of doxorubicin [3, 4]. Telmisartan, an angiotensin II receptor blocker (ARB) is used for the treatment of hypertension and cardiovascular diseases and is now also known to possess anticancer potential [5]. Aside from its typical function in the renin-angiotensin system, telmisartan is a partial agonist of the peroxisome proliferator-activated receptor gamma (PPAR-γ), a nuclear receptor central to inflammation and cellular differentiation and proliferation. This combined activity may position telmisartan as an attractive candidate for drug repurposing in oncology; especially as accumulating data is available that PPAR-γ activation might hinder tumor growth and sensitize cancer cells to chemotherapeutics. [6, 7].

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Recent reports indicated the existence of a synergistic effect between doxorubicin and telmisartan on several cancer cell lines. While the exact mechanisms behind this increase in cytotoxicity are still unknown, several factors have already been described. Suggested mechanisms of action are reduction of oxidative stress, downregulation of pro-survival signaling pathways such as PI3K/Akt and NF- κ B, change in mitochondrial membrane potential, and the promotion of intracellular doxorubicin retention by inhibition of efflux transporters like P-glycoprotein and BCRP [8, 9]. Furthermore telmisartan may influence the tumour microenvironment and thereby decrease angiogenesis and inflammation, which may also explain its chemosensitizing properties [10]. The objective of this study was to investigate the mechanism(s) of telmisartan's enhancing activity on doxorubicin-induced cytotoxicity on some cancer cells. Using an approach that includes dissection of cellular responses such as apoptosis, cell cycle arrest, and alterations in gene and protein expression, we plan to elucidate the molecular foundation of this interaction. Knowing these mechanisms is essential not only for discovering new combinations of drugs, which may enhance treatment effectiveness and decrease toxicity in cancer patients. Additionally, a strategy of re-purposing an established cardiovascular medicine with a proven safety profile offers a more cost and time-effective opportunity for improving existing cancer treatments.

Methodology

2.2: Place of the study This study was carried out at Iraq Biotech Company for Scientific Research in Basrah at Iraq, during the period from January to May, 2024. It was designed to assess the cytotoxic activity against the A549 human lung carcinoma cell line, and it was divided into control (non-treated), Doxorubicin-treated, Telmisartan-treated, and combination-treated (1:1) groups. All cells were treated with six concentrations (800, 400, 200, 100, 50 and 25 μ g/mL) for 72 h. The solutions in the work (Trypsin, EDTA, PBS, MTT and the antibiotics: penicillin and streptomycin) were all addressed according to the standard procedures, and have been properly sterilized and saved. The culture medium (RPMI-1640) was supplemented with fetal bovine serum and antibiotics, along with serum-free medium and freezing medium for specific procedures. A549 cells were thawed, cultured under standard incubator

conditions (37°C, 5% CO₂, 95% humidity), and subcultured when they reached 70% confluency. Cells were harvested using trypsin-EDTA, and either used for assays or cryopreserved. Cell viability was assessed using Trypan blue exclusion and a hemocytometer before treatment application. The MTT assay was used to assess cytotoxicity across different treatment groups.

Statistical analysis

Data were presented as means $\pm$ S.D. (Standard deviation), gathered and analyzed utilizing GraphPad Prism Edition 10 and Microsoft Office Excel 2019. The Post-Hoc-Tukey test was used for multiple comparisons between all paired combinations in the study groups, after the one-way ANOVA (Analysis of Variance) test was used to evaluate statistically significant differences in mean values among various groups. When studying the cancer cell line, differences between tested medicines were assessed using a two-way ANOVA test. IC₅₀ was computed using GraphPad Prism 10, and CI and DRI were computed using Compusyn software. A P-value of less than 0.05 was considered statistically significant.

Ethical approval

The study was approved by the human ethics committee of Department of Pharmacology, College of Medicine, University of Basrah, Basrah, Iraq.

Results

Effect of Doxorubicin and Telmisartan on Cell Viability at Different Concentrations

The results in the Table 1, showed a significant increase in the percentage of cell inhibition with increasing doxorubicin concentration, with lower rates when treated with doxorubicin or telmisartan alone compared to the combined treatment. The combined treatment group demonstrated a significantly increased cell inhibition effect at all concentrations, reaching 86.78% at 800 mg/ml compared to treatment with either compound alone. The differences between the three groups were statistically significant at most concentrations, indicating a synergistic effect between the two drugs that significantly enhanced the cell inhibition efficacy compared to the individual and control treatments.

Table 1: Percentage of Cell Viability (% $\pm$ SD) after Treatment with Doxorubicin Alone, Telmisartan Alone, and Their Combination

Doxorubicin Conc. (mg/ml)	Doxorubicin Alone (% $\pm$ SD)	Telmisartan Alone (% $\pm$ SD)	Doxorubicin + Telmisartan (% $\pm$ SD)	Significant Differences
Control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	—
25	10.11 $\pm$ 0.47	9.62 $\pm$ 5.84	51.21 $\pm$ 5.09	a, b, c
50	11.22 $\pm$ 1.27	11.01 $\pm$ 8.21	52.86 $\pm$ 9.69	a, b, c
100	18.75 $\pm$ 6.44	13.76 $\pm$ 4.54	60.34 $\pm$ 10.19	a, b, c
200	27.98 $\pm$ 7.03	22.54 $\pm$ 5.20	76.09 $\pm$ 6.57	a, b, c
400	50.99 $\pm$ 5.64	51.56 $\pm$ 8.22	81.91 $\pm$ 3.90	a, b, c
800	63.72 $\pm$ 7.35	71.95 $\pm$ 2.73	86.78 $\pm$ 2.02	a, b

a: Significant vs. Doxorubicin alone, b: Significant vs. Telmisartan alone, c: Significant vs. Control

Enhanced Effect of Combined Doxorubicin and Telmisartan Treatment on Cell Viability

The Table 2, shows the percentage increase in cell inhibition with the combination treatment compared to doxorubicin or telmisartan alone. The highest percentage increase was observed at lower concentrations, exceeding 400% at 25 mg/ml compared to either drug alone. As the concentration

increased, the percentage increase gradually decreased, reaching 36.19% compared to doxorubicin and 20.62% compared to telmisartan at 800 mg/ml. These results reflect a strong synergistic effect of the combination treatment in improving cell inhibition, especially at lower concentrations, suggesting the importance of combining the two drugs to enhance therapeutic efficacy.

Table 2: Percentage Increase in Cell Inhibition Compared to Doxorubicin Alone and Telmisartan Alone

Doxorubicin Conc. (mg/ml)	↑ Over Doxorubicin Alone (%)	↑ Over Telmisartan Alone (%)
25	+ 406.58%	+ 432.37%
50	+ 370.93%	+ 380.11%
100	+ 221.81%	+ 338.36%
200	+ 171.99%	+ 237.55%
400	+ 60.63%	+ 58.82%
800	+ 36.19%	+ 20.62%

Increase (%) = $\frac{(\text{Combination} - \text{Monotherapy})}{\text{Monotherapy}} \times 100$

Increase (%) = $\frac{(\text{Combination} - \text{Monotherapy})}{\text{Monotherapy}} \times 100$

Cytotoxicity and Synergistic Effects of Doxorubicin and Telmisartan Combination

The results in Table 3, showed that the 50% inhibitory concentration (IC₅₀) of the combined treatment (72.21 µg/ml) was significantly lower compared to the use of doxorubicin (292.46 µg/ml) or telmisartan (341.97 µg/ml) alone, indicating increased efficacy. The interaction coefficient (CI) also showed a value of 0.45, indicating a synergistic effect between the two drugs in the combined treatment. Moreover, the high DRI of doxorubicin (8.11) and telmisartan (9.47) indicates that a lower dosage of drugs is necessary in case the combined treatment is employed, increasing efficacy and decreasing unwanted effects. These findings indicate that the doxorubicin/telmisartan-based therapy is a potential novel therapeutic approach for greater anticancer efficacy.

Table 3: IC₅₀, Combination Index (CI), and Dose Reduction Index (DRI) Values for Treatments

Parameter	Doxorubicin Alone	Telmisartan Alone	Combination Treatment
IC ₅₀ (µg/ml)	292.46	341.97	72.21
CI	—	—	0.45 (Synergism)
DRI	8.11	9.47	—

CI < 1 indicates synergistic interaction; DRI > 1 indicates favorable dose reduction in combination therapy

Cytotoxicity Levels of Doxorubicin, Telmisartan, and Their Combination

As represented, the average cytotoxicity in the combined therapy group was significantly higher (68.36%±12.49) than that in the doxorubicin alone (30.48%±20.34) or telmisartan alone (30.41%±23.10) group 26. The range of toxicity with the combination therapy was 51.21% to 86.78% and compares with higher efficacy and stability to individual treatments, with toxicity rates of 9.62% to 71.95%. These results suggest that combined treatment of doxorubicin with telmisartan markedly increases the cytotoxic effect, Table 4.

Table 4: Range and Mean Cytotoxicity Percentages with Standard Deviation for Treatment Groups

Treatment Group	Cytotoxicity Range (%)	Mean Cytotoxicity ± SD (%)
Doxorubicin Alone	10.11-63.72	30.48±20.34
Telmisartan Alone	9.62-71.95	30.41±23.10
Combination Therapy	51.21-86.78	68.36±12.49

Doxorubicin effect on BCL-2 Concentration

The findings of the study showed a significant decrease in BCL-2 concentration (P=0.0229) post treatment of A549 cells with IC₅₀ of Doxorubicin compared to the control group, as shown in Figure 1.

Telmisartan effect on BCL-2 Concentration

The study results showing a significant decrease in BCL-2 concentration (P= 0.0458) following the treatment of A549 cells with the IC₅₀ of Telmisartan when compared to the control group, as per the results shown in Figure 1.

Comparison between the effect of Doxorubicin plus Telmisartan combination against control group on the BCL-2 Concentration

Treating A549 with Doxorubicin plus Telmisartan combination IC₅₀ showed a reduction in BCL-2 level but this reduction was not significant (P= 0.1671) when compared to control group, as shown in Figure 1.

Comparison between the effect of Doxorubicin alone and against Doxorubicin plus Telmisartan combination on the BCL-2 Concentration

Subjecting A549 to Doxorubicin plus Telmisartan combination IC₅₀ showed a reduction in BCL-2 level but this reduction was not significant (P= 0.1290) compared to cells treated to Doxorubicin alone, as shown in Figure 1.

Comparison between the effect of Telmisartan and Doxorubicin on the BCL-2 Concentration

There was no significant difference was detected between Telmisartan and Doxorubicin-treated cells, as seen in Figure 1.

Comparison between the effect of Doxorubicin plus Telmisartan combination against Telmisartan on the BCL-2 Concentration

Treating A549 with Doxorubicin plus Telmisartan combination IC₅₀ showed a reduction in BCL-2 level but this reduction was not significant (P= 0.3048) when compared to Telmisartan group, as shown in Figure 1.

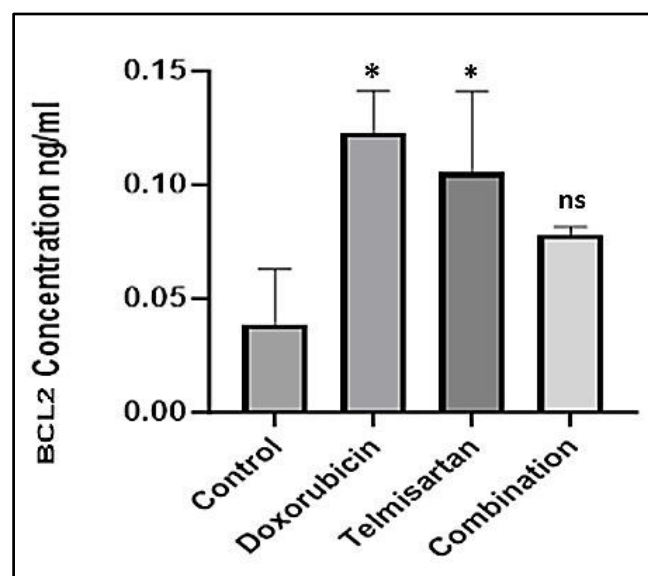


Fig 1: Statistical analysis (One-Way ANOVA), Effects of Doxorubicin, Telmisartan and their combination on BCL-2 concentration in the A549 cell line. * P < 0.05, ns P > 0.05 compared to control.

Doxorubicin effect on Caspase 3 Concentration

The findings of the study showed a significant Increase in caspase 3 concentration ($P = 0.0096$) after treatment of A549 cells with IC_{50} of Doxorubicin compared to the control group, as shown in Figure 2.

Telmisartan effect on Caspase 3 Concentration

The study results showing a significant Increase in caspase 3 ($P = 0.0011$) following the treatment of A549 cells with the IC_{50} of Telmisartan when compared to the control group, as per the results shown in Figure 2.

Comparison between the effect of Doxorubicin plus Telmisartan combination against control on the caspase 3 concentration

Subjecting A549 cells to Doxorubicin plus Telmisartan combination IC_{50} Increase in caspase 3 level, but this increase was not significant ($P = 0.2447$) compared to control, as shown in Figure 2.

Comparison between the effect of Doxorubicin plus Telmisartan combination against Doxorubicin alone on the caspase 3 concentration

Subjecting A549 cells to Doxorubicin plus Telmisartan combination IC_{50} showed Increase in caspase 3, but this increase was not significant compared to cells treated to Doxorubicin alone, as shown in Figure 2.

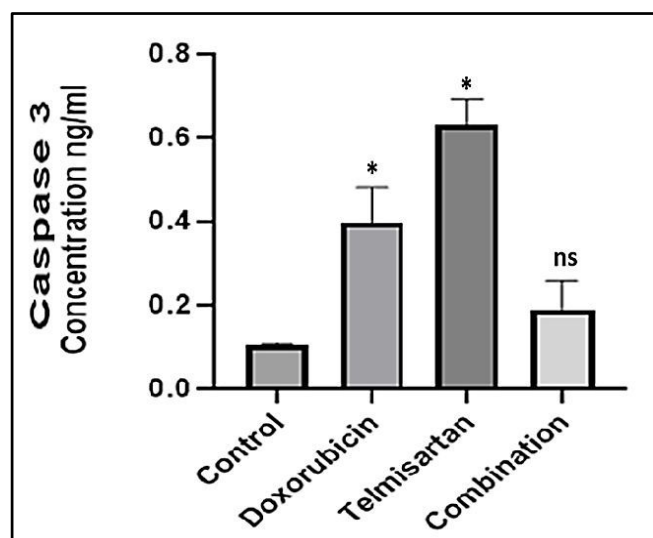


Fig 2: Statistical analysis (One-Way ANOVA): Effects of Doxorubicin, Telmisartan and their combination on Caspase 3 concentration in the A549 cell line. * $P < 0.05$, ns $P > 0.05$ compared to control.

Discussion

In this study, we evaluated the cytotoxic and apoptotic effects of Doxorubicin, Telmisartan, and Doxorubicin-Telmisartan on the A549 cancerous cell line. The cytotoxicity results displayed a profound synergistic action of Doxo when used in combination with Tel compared to single drug at various ranges of concentration. For example, the combination of the two agents at 800 $\mu\text{g/ml}$ resulted in 86.78% cytotoxic effect which was higher than that for Doxorubicin (63.72%) and Telmisartan (71.95%) when the two were given separately. The observed results were also substantiated by the IC_{50} values that depicted a significantly lower IC_{50} (72.21 $\mu\text{g/ml}$) of combination treatment

compared to Doxorubicin (292.46 $\mu\text{g/ml}$) or Telmisartan (341.97 $\mu\text{g/ml}$) alone. Furthermore, the combination showed a low combination index ($CI = 0.45$) point to strong synergism, and high of drug reduction indices (DRI) for both agents, which suggested high possibility of an increase of the therapeutic efficiency at low doses [10, 11]. Apoptotic markers were measured to shed light on the mechanisms of cytotoxicity. Caspase-3 concentration increased significantly after single treatment with Doxorubicin or Telmisartan. Consistent with this result, the combination group also exhibited upregulation of caspase-3, but no significant difference was seen as compared to control or single groups [12]. This possible elevated plateau of apoptotic induction when both the drugs are combined could be indicative of other additional cell death mechanisms [13, 14]. The anti-apoptotic marker BCL-2 was found to be significantly decreased in the Doxorubicin as well as Telmisartan treated groups. However, for combined treatment, statistical difference between combined and either control or monotherapy was not observed [15, 16]. This may indicate that the apoptotic signaling induced by the combination regimen is not at the level of BCL-2 regulation significantly more effective than that of the single agents. Second, the increased cytotoxicity may not be solely attributable to an inhibition of BCL-2-induced apoptosis, but due to additional signaling pathways as oxidative stress, cell cycle arrest, or autophagy [17, 18]. Curiously, although effect of each drug on apoptotic markers was prominent, their combination failed to potentiate it significantly, in spite of a stronger cytotoxic response [19, 20]. This disparity may suggest a complex interaction of mechanisms beyond mere additive impact on apoptosis. The distinct regulation of apoptotic mediators in combination group could imply that, the cellular response could be multi-factorial, which needs to be addressed further at the genomic level and comprehensive pathway analyses [21].

Conclusion

In sum, the combination of Doxorubicin and Telmisartan exerts a potent cytotoxic synergy against A549 cells during which IC_{50} values are sharply declined and cell death is remarkably intensified. The increased apoptotic markers such as BCL-2 reduction and caspase-3 activation in combination group were not unequivocally evidenced, however, and further mechanistic studies in this respect are warranted. The findings lend support to the notion that the combination of antihypertensive agents such as Telmisartan, with chemotherapeutic agents, may prove to be effective in achieving higher anticancer effectiveness with potential dose-sparing effects.

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