

Analysis of the cellular viability and migratory capacity of triple-negative breast cancer cells stimulated with doxorubicin used during neoadjuvant chemotherapy

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Abstract— Breast cancer remains a major public health problem, especially the triple-negative molecular subtype, due to its limited response to chemotherapy. Therefore, the objective of this study was to evaluate the cellular viability and migratory capacity of triple-negative breast cancer cells treated with doxorubicin, a drug used in neoadjuvant chemotherapy. For this purpose, the MDA-MB-231 cell line was used, cultured in DMEM medium supplemented with 10% fetal bovine serum and 1% antibiotic. Response to treatment was evaluated using MTT, clonogenic, and wound healing assays.

The results showed an IC_{50} value of 0.3 μ M for doxorubicin in the MDA-MB-231 cell line. It was also observed that, despite treatment, the cells retained partial migratory capacity. Taken together, these findings indicate that doxorubicin reduces cell viability but does not completely inhibit the migration of MDA-MB-231 cells, which could contribute to doxorubicin favoring the survival of this triple-negative molecular phenotype.

Keywords— Doxorubicin, breast cancer, cell migration, clonogenic assay, cell survival.

I. INTRODUCTION

Breast cancer continues to be one of the leading causes of death in women worldwide, representing a major public health problem due to its high incidence, clinical diversity, and biological complexity. This disease is characterized by marked molecular heterogeneity, which translates into a variable response to chemotherapy treatments and significant differences in patient prognosis [1]. Within this context, triple-negative breast cancer (TNBC) is a subtype with a molecular profile of particular clinical relevance, defined by the absence of estrogen, progesterone, and HER2 receptor expression, which considerably limits targeted therapeutic options and is associated with highly aggressive behavior, a high recurrence rate, and greater metastatic potential [2].

In this scenario, the study of representative cell models is crucial for understanding the biological mechanisms underlying tumor progression and the response to treatment. The MDA-MB-231 cell line is widely used as an in vitro model of triple-negative breast cancer due to its mesenchymal phenotype, high migratory and invasive capacity, and relative resistance to various conventional therapies, making it a key tool for analyzing processes such as cell viability and tumor migration [3].

On the other hand, doxorubicin is a chemotherapeutic agent belonging to the anthracycline group widely used in the treatment of breast cancer, particularly in neoadjuvant chemotherapy regimens, with the aim of reducing tumor mass before surgery, improving tumor respectability, and decreasing the risk of disease recurrence [4]. Its classic mechanism of action involves DNA intercalation, inhibition of topoisomerase II, and generation of reactive oxygen species, leading to genetic damage and cell death. However, recent evidence indicates that doxorubicin's biological effects extend beyond its cytotoxic properties, suggesting it may influence additional cellular pathways or processes [4].

Several studies have suggested that this drug may induce the activation of molecular signaling pathways associated with inflammatory processes, cell survival mechanisms, and tumor progression, highlighting the TLR4/NF- κ B axis as one of the key pathways involved [5]. Sustained activation of these pathways has been linked to the regulation of genes involved in drug resistance, apoptosis evasion, and tumor microenvironment remodeling. In particular, NF- κ B-mediated signaling has been linked to the induction of epithelial-mesenchymal transition (EMT), a fundamental biological

process in the acquisition of a more migratory and invasive phenotype, favoring the metastatic spread of breast cancer cells, especially in triple-negative models such as the MDA-MB-231 cell line [6].

Despite advances in the clinical management of breast cancer, it is estimated that approximately 20% of patients do not respond adequately to neoadjuvant chemotherapy, resulting in a lower complete pathological response rate and an unfavorable prognosis [7]. This therapeutic resistance highlights the need to further study the cellular and molecular effects induced by widely used drugs, such as doxorubicin. This is especially important in aggressive subtypes such as triple-negative breast cancer.

In this context, the present study aims to evaluate the impact of doxorubicin on the viability and migratory capacity of the MDA-MB-231 cell line, with the purpose of providing experimental evidence that contributes to a better understanding of its biological behavior in response to chemotherapy treatment. The results obtained could provide relevant information for optimizing therapeutic strategies and developing more effective approaches to treating triple-negative breast cancer.

II. METHODOLOGY

A. Cell Culture

The MDA-MB-231 breast cancer cell line was cultured and maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution. For *in vitro* assays, cells were incubated and monitored periodically until they reached a confluence of no more than 80% [8], [9].

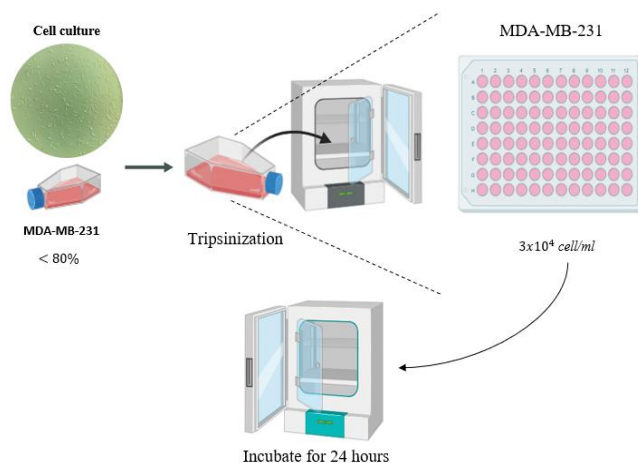


Figure 1. Cell Culture Methodology

B. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide)

The MTT reduction assay is a widely used colorimetric method for assessing cell viability and metabolic activity. This assay is based on the ability of metabolically active cells to reduce the compound 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to insoluble purple-colored formazan crystals. This reaction is mainly catalyzed by NAD(P)H-dependent oxidoreductase enzymes located in the mitochondria, although other cytosolic enzymes may also participate. The amount of formazan generated is directly proportional to the number of viable cells present in the sample. After incubation with MTT, the formazan crystals are solubilized using an organic solvent, allowing their spectrophotometric quantification, generally at a wavelength between 540 and 570 nm. A decrease in absorbance indicates a reduction in cell viability, while high values reflect increased metabolic activity.

Due to its simplicity, reproducibility, and sensitivity, the MTT assay is widely used in cytotoxicity studies, cell proliferation studies, and the evaluation of the efficacy of pharmacological compounds *in vitro*.

For cell viability analysis, cells were cultured in 96-well plates at a cell density of 3×10^4 cells/mL, then treated with serial concentrations of doxorubicin (initial concentration of $10 \mu\text{M}$) for 24 hours. After the incubation period, MTT reagent was added to a final concentration of 0.5 g/mL, and the plates were incubated at 37°C with 5% CO_2 for 3 hours. Once incubation was complete, the supernatants were removed, and dimethyl sulfoxide (DMSO) was added to dissolve the formazan crystals. Absorbance readings were taken at 550 nm. The IC_{50} value was determined by nonlinear regression analysis using GraphPad Prism software [10], [11].

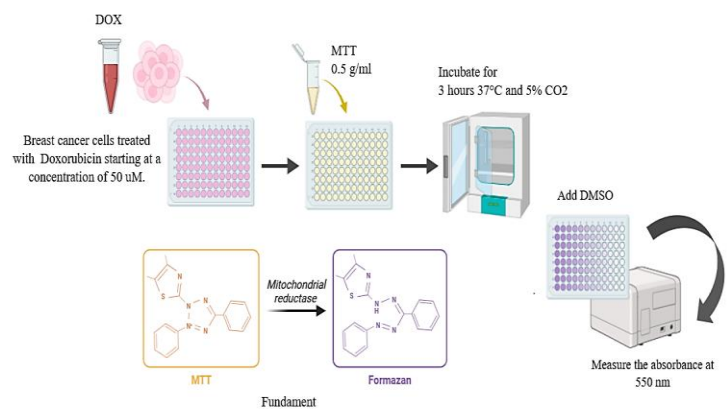


Figure 2. MTT assay methodology

C. Clonogenic Assay

The MDA-MB-231 breast cancer cell line was seeded in 6-well plates (500 cells per well) in complete DMEM medium. After 24 hours of incubation, the cells were treated with the IC_{50} concentration of doxorubicin ($0.3 \mu M$) and maintained at $37^{\circ}C$ with 5% CO_2 . After 24 hours of treatment, the culture medium was replaced, and the time required for colony formation to begin was monitored. Once this stage was reached, the colonies were fixed and stained with 0.5% crystal violet. Finally, the stained slides were observed under a stereoscope [12].

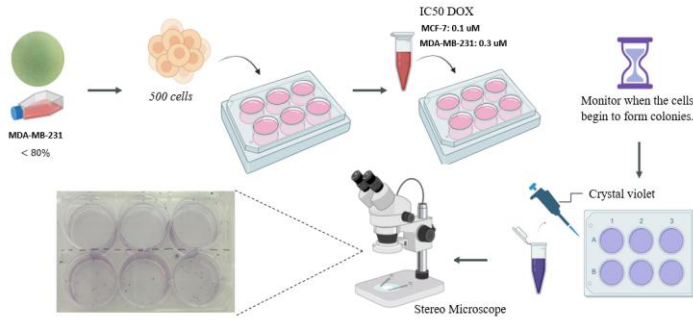


Figure 3. Clonogenic assay Methodology

D. Wound Healing test

MDA-MB-231 cells were cultured in 24-well plates until they reached less than 90% confluence in complete DME medium. Once the required convergence was achieved, a linear wound was created using a sterile $200 \mu L$ tip, making a uniform incision at the bottom of each well to standardize the opening of the "scar."

After 24 hours, the concentration equivalent to the IC_{50} ($0.3 \mu M$) of doxorubicin was added, and subsequently, the plates were placed under an inverted microscope, and images of the wound area were captured at 24, 48, and 72 hours, allowing for monitoring of scar closure and the migratory behavior of the treated cells [13]. Finally, the remaining wound area was quantified using ImageJ software.

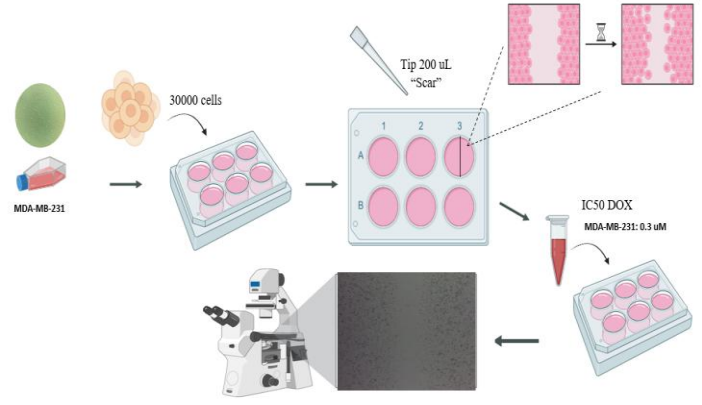


Figure 4. Wound Healing Test Methodology.

III. RESULTS

A. DETERMINATION OF THE IC_{50}

The mean inhibitory concentration (IC_{50}) of doxorubicin in the MDA-MB-231 cell line was determined from a dose-response assay using 12 serial concentrations, starting at $50 \mu M$. Data analysis revealed an IC_{50} value of $0.3 \mu M$, indicating that this concentration of doxorubicin reduces the cell viability of the MDA-MB-231 line by 50%.

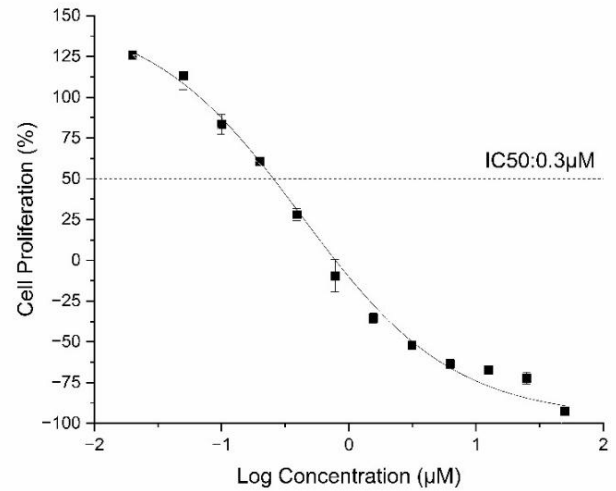


Figure 5. Dose-response curve of the MDA-MB-231 cell line treated with doxorubicin.

B. Clonogenic Assay

Figures 6 and 7 show that untreated MDA-MB-231 cells (control) exhibited a high capacity for colony formation. In contrast, treatment with the IC_{50} concentration of doxorubicin ($0.3 \mu M$) produced a marked decrease in both the number and

size of the colonies formed. These results show that doxorubicin significantly affects cell survival and reduces the clonogenic potential of the MDA-MB-231 cell line.

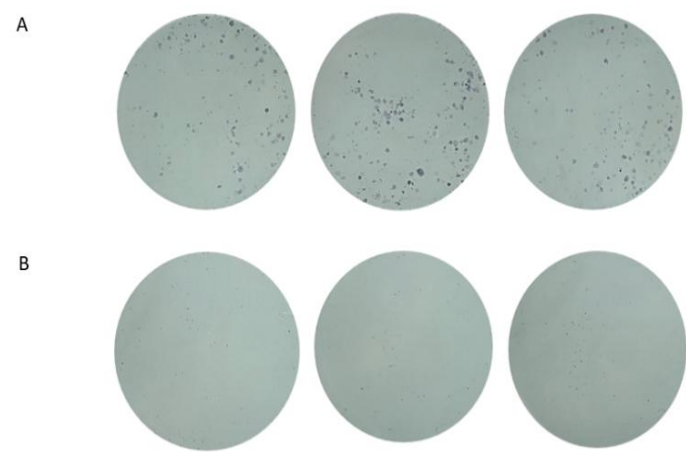


Figure 6. Clonogenic capacity of MDA-MB-231 cells stimulated with doxorubicin. Where A: are untreated cells (control) and B: are cells stimulated with doxorubicin.

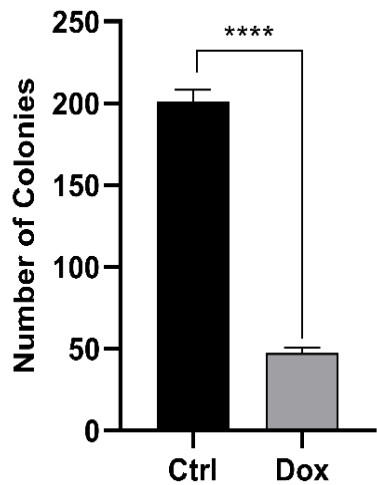


Figure 7. Quantitative analysis of the clonogenic assay in MDA-MB-231 cells stimulated with doxorubicin.

C. Wound Healing Test

Three experimental conditions were evaluated: negative control (C–, DMEM with 2% SBF), positive control (C+, DMEM with % SBF), and cells treated with doxorubicin (Dox, IC50), analyzed at different incubation times. Treatment with doxorubicin at 24 and 48 hours showed a significant increase in

migratory capacity compared to controls ($p > 0.05$). However, at 72 hours, there was evidence of partial recovery of the healing process below the control groups.

Taking together, these results indicate that MDA-MB-231 cells partially maintain their migratory capacity, suggesting that this drug does not completely inhibit migratory behavior and, on the contrary, may be promoting cell migration at 24 and 48 hours.

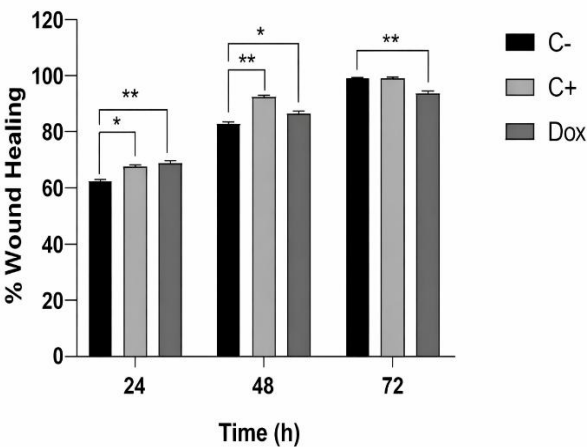


Figure 8. Percentage of healing in MDA-MB-231 cells evaluated at 24, 48, and 72 h.

IV. DISCUSSION

Several studies have reported IC₅₀ values for doxorubicin in the MDA-MB-231 cell line within a range of approximately 0.2 to 1 μM, a variability that can be attributed mainly to differences in experimental designs, such as exposure time, initial cell density, the type of viability assay used, and cell culture conditions. In this context, Pilco-Ferreto and Calaf (2016) determined an IC₅₀ of 1 μM after 48 h of exposure to doxorubicin, while Razak Abd et al. (2019), when evaluating the cytotoxicity of eupatorine *in vitro* using doxorubicin as a positive control, reported an IC₅₀ of 0.7 μM after 48 h of incubation [14], [15].

The present study shows that doxorubicin has an IC₅₀ of 0.3 μM in MDA-MB-231 cells, a value that falls within the range previously described in the literature. This finding confirms that doxorubicin retains marked cytotoxic efficacy against this triple-negative breast cancer model, reflecting a high affinity of the drug for tumor cells and a significant cytotoxic response even at relatively low concentrations. Furthermore, this result suggests a high initial sensitivity of MDA-MB-231 cells to doxorubicin, which is consistent with its mechanism of action

based on the induction of DNA damage and the generation of oxidative stress.

In relation to clonogenic capacity, treatment with doxorubicin produced a significant decrease in colony formation in MDA-MB-231 cells, demonstrating a sustained long-term cytotoxic effect beyond the initial inhibition of cell viability. This result is particularly relevant, as the clonogenic assay assesses the ability of a tumor cell to survive, proliferate, and form colonies after exposure to a cytotoxic agent, representing a key indicator of residual tumorigenic potential. In this regard, our findings are consistent with those reported by Romaniuk-Drapała et al. (2021), who demonstrated that doxorubicin drastically reduces colony-forming capacity in various breast cancer lines, including MDA-MB-231, by altering DNA repair mechanisms and inhibiting cell proliferation [16].

Similarly, Bojko et al. (2020) described that exposure to doxorubicin induces cellular senescence and decreases clonogenic survival, negatively affecting long-term cell viability [17]. Taken together, these results reinforce the idea that doxorubicin not only exerts an acute cytotoxic effect but also compromises the future proliferative capacity of tumor cells, which is essential from a therapeutic perspective to prevent tumor relapse. However, when evaluating migratory capacity using the healing assay, differential behavior was observed. MDA-MB-231 cells treated with doxorubicin showed an initial increase in wound closure at 24 and 48 h compared to the negative control; however, at 72 h, the percentage of closure was similar to that observed in the control group. This pattern suggests that, despite reduced viability and clonogenic capacity, surviving cells retain or even temporarily enhance their migratory capacity in the early stages after exposure to the drug.

This behavior is consistent with that described in the study Quercetin Enhances the Suppressive Effects of Doxorubicin on the Migration of MDA-MB-231 Breast Cancer Cells (2021), which showed that doxorubicin alone has only a partial anti-migratory effect on this highly aggressive and metastatic cell line. These authors demonstrated that only the combination of doxorubicin with other bioactive agents, such as quercetin, achieves significant inhibition of cell migration, suggesting the activation of compensatory mechanisms in response to chemotherapeutic stress [18].

Taken together, these findings indicate that, although doxorubicin exhibits effective cytotoxic activity and significantly reduces the clonogenic capacity of MDA-MB-231

cells, it is insufficient to completely suppress cell motility in this triple-negative breast cancer model and may even temporarily promote this migratory behavior. This phenomenon could be associated with intrinsic mechanisms of tumor plasticity, as well as the activation of survival and pro-migratory signaling pathways, such as the TLR4/NF- κ B axis, the induction of EMT, and cell reprogramming induced by chemotherapy treatment. These cellular adaptations could contribute to the maintenance of metastatic potential and therapeutic resistance observed clinically in patients with triple-negative breast cancer.

V. CONCLUSION

This study evaluated the cell viability and migratory capacity of triple-negative breast cancer cells treated with doxorubicin. The results showed that doxorubicin has an IC₅₀ value of 0.3 μ M, and complementarily, the clonogenic assay showed a significant decrease in clonogenic capacity in treated cells, indicating an inhibitory effect on long-term cell survival. However, in the wound healing assay, an increase in wound closure was observed at 24 and 48 hours compared to the negative control, suggesting that, despite its cytotoxic effect, doxorubicin may be promoting survival and migration mechanisms in this subtype of triple-negative breast cancer.

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