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Investigating the Effects of Combining Inorganic Nanoparticles with Doxorubicin on Human Breast Cancer MCF-7 Cells

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INTRODUCTION

Breast cancer remains the most diagnosed malignancy in women worldwide. Despite advancement in cancer therapeutics, the use of adjuvant chemotherapy remains the cornerstone treatment approach for breast cancer. However, the increased number of women presenting to clinics with metastatic disease or serious adverse effects remains a challenge. Therefore, new treatment approaches are urgently needed to enhance the therapeutic efficacy and prevent un-tolerated toxicities of chemotherapeutic medications. The biomedical applications of inorganic nanoparticles (inorganic NPs), including their potential anti-tumor activity, have increased dramatically in the last twenty years. The aim of our study was to investigate how individual and combination treatments with Dox and inorganic NPs affect cell viability and proliferation, redox status, and mechanism of cell death, using MCF-7 human breast cancer cells as an *in vitro* model. We hypothesized that pretreatment with subtoxic concentrations of inorganic nanoparticles (ZnO NPs and NiO NPs) sensitize the Human Breast Cancer MCF-7 Cells to DOX.

OBJECTIVES

A. To determine the extent of cytotoxic effects of combining DOX with inorganic nanoparticles on the MCF-7 breast cancer cell line.

B. To determine the mechanism by which the combination intervention induces cell death.

C. To evaluate the role of mitochondria in the MCF-7 response to DOX-nanoparticles combination.

METHODS

Human breast adenocarcinoma (MCF-7) cells was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, and 1% penicillin/streptomycin under standard culture conditions (5% CO_2, 95% humidity, and 37°C). All treatment conditions were in a serum-free medium. In combination therapy, a subtoxic concentrations of NPs were added to cells before the DOX treatment.

1. MTT Assay: 10,000 MCF-7 cells per well were seeded in 96-well plates, incubated overnight, then treated for 48 hrs with different doses of Dox, ZnO NPs, or NiO NPs to determine their IC50. In the case of the combination therapy, cells were treated with IC50 doses of the ZnO NPs or NiO NPs for 24h, then we changed the media with different doses of DOX in serum-free medium for another 24h before performing staining with MTT.

2. Annexin V/PI & Mitochondrial Membrane Potential Assays: MCF-7 cells were seeded at a density of 2.5×10^5 cells per 35mm dishes and incubated for 24 hrs at 5% CO_2, 95% humidity, and 37°C. Cells then were either left untreated in a 10% DMEM, left untreated and deprived of serum for 48 hrs, treated with Dox or ZnO NPs for 48 hrs, or treated with ZnO NPs for 24 hrs then DOX for another 24hrs. After that, cells were either trypsinized or scraped and harvested in 1.5 Eppendorf tubes and centrifuged at 3000 RPM for 10 min. the supernatant were discarded and the pellet were washed with PBS. After another round of centrifugation and washing with PBS, pellets were resuspended and stained with AnnexinV-PI staining (For Apoptosis) or with TMRM (For mitochondrial membrane potential) and incubated for 30 minutes at room temperature (for Annexin V-PI assay) or 37°C (For TMRM). Both assays were detected and analyzed using a flow cytometer.

RESULTS

Figure 1A. Inorganic Nanoparticles Dose-Response Curve
ZnO was almost five times more potent than NiO.

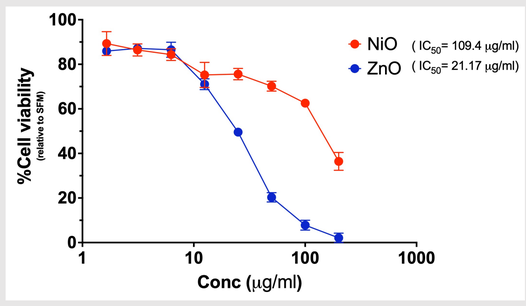
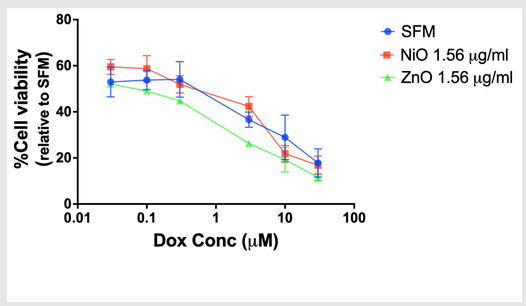


Figure 1B. Combination therapy Dose-Response Curve
MCT-7 Cells pre-exposed to subtoxic concentration of ZnO were more sensitive to DOX treatment.



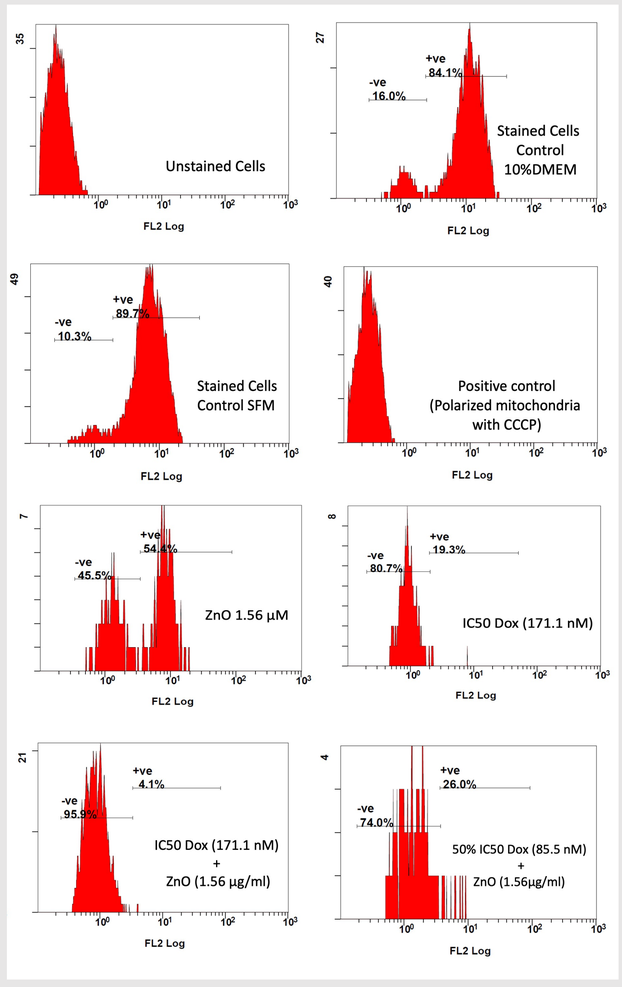


Figure 2. Mitochondrial Membrane Potential (TMRM) Assay
ZnO NPs exacerbated DOX-mediated mitochondrial dysfunction in MCF-7. TMRM accumulation in mitochondria is driven by depolarized mitochondrial membrane. The shift in TMRM fluorescence to the left indicate failure of dye to incorporate due to potential collapse in case of apoptosis (membrane polarization).

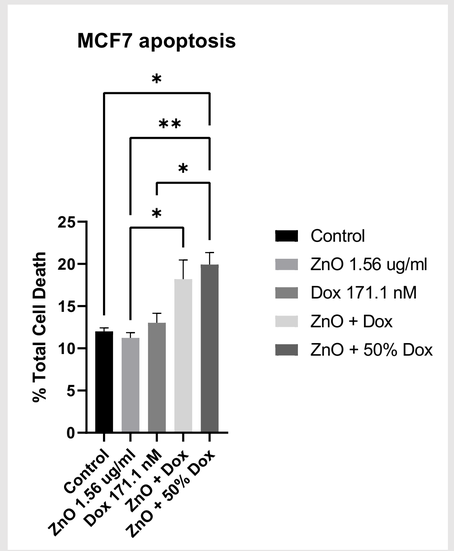


Figure 3. Annexin V/PI Apoptosis Assay
Preexposure to subtoxic concentration of ZnO significantly increased the percentage of MCF-7 cell population undergoing apoptosis

CONCLOSIONS

- **Our results suggest that ZnO NPs sensitized MCF-7 human breast cancer cells to DOX treatment.**
- **ZnO NPs-DOX treated MCF-7 cells displayed apoptotic cell death features.**
- **The mitochondrial membrane polarization could suggest mitochondrial dysfunction**
- **Future experiments will assess how mitochondrial dysfunction initiate the cascade of events in MCF-7 cells treated with ZnO NPs and DOX.**

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