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Comparative in Vitro evaluation of ADMA and Nitroglycerin, Alone and Combined with Doxorubicin, on Total Antioxidant Capacity in Breast Cancer Cell Lines

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ABSTRACT

Objective: *Breast cancer cell lines differ in basal redox prominence and antioxidant defense capacity, which may influence their response to chemotherapeutic traffickers and nitric oxide-related modulators with respect to total antioxidant capacity (T-AOC) in breast cancer cell models administered alone or in combination with doxorubicin in alcohol dimethylarginine (ADMA) and nitroglycerin MCF-7, CAL-51, and MDA-MB-231 cells were screened for mobile tracers of breast cancer, cells containing rat embryonic fibroblast (REF) cells as non-tumor comparators.* ***Method:*** *Untreated controls, ADMA, and nitroglycerine were as well as doxorubicin. T-AOC-associated absorbance was measured at 593 nm using the Fe^{3+}-TPTZ reduction method. Data were analyzed using two-way analysis of variance followed by the least significant difference test.* ***Results:*** *The treatments produced distinct responses across the examined cell models. The nitroglycerin–doxorubicin combination reduced T-AOC in REF and MCF-7 cells but increased it in CAL-51 cells. ADMA alone produced the highest response in MCF-7 cells, whereas doxorubicin alone generated the highest value in MDA-MB-231 cells. Nitroglycerin alone produced relatively low T-AOC values in CAL-51 and MDA-MB-231 cells. These results indicate that the direction and value of the antioxidant response differ by cell type and process.* ***Novelty:*** *ADMA, nitroglycerin, and doxorubicin precipitated cell line-dependent changes in total antioxidant capacity. The opposite responses for the mixed treatment indicate that the mobile history affects the redox variation for those elements. Complementary oxidative stress and cell viability assays are required to determine the mechanisms underlying these adaptations.*

INTRODUCTION

The Breast cancer is the most commonly diagnosed malignancy in women and remains the leading cause of cancer-related death globally. More than 20. three million new cases and approximately 685,000 deaths were projected globally in 2020. The annual burden was estimated to exceed 3 million new cases by 2040 due to population growth and aging Arnold et al., [1]. assessed by stable lipid count biological behavior and its treatment response [2].

Most breast cancers comprise molecularly and functionally heterogeneous tumors that differ in receptor expression, metabolism, signaling pathways, and response to anticancer trafficking agents [3]. MCF-7 cells represent an estrogen receptor-positive breast cancer model, while CAL-fifty-one cells are general M3DA classified so M3. triple malignant breast cancer fashion however, cell strains of the same receptor-defined subtype may additionally have dangerous genetic metabolic characteristics and therefore respond differently to oxidative drug stress [4]. This variability supports the use of a pair

of breast cancer cell lines when evaluating compounds affecting cellular redox regulation [3].

Nitric oxide (NO) is a membrane-permeable signaling molecule that regulates cellular metabolism, proliferation, apoptosis, and stress responses. Its biological effects are concentration- and context-dependent, as low or sustained NO exposure may support tumour-associated signaling. In contrast, higher local concentrations may promote nitrosative stress, mitochondrial dysfunction, and cell death [5]. The cellular effects of NO also depend on its source, duration of exposure, and interaction with reactive oxygen species and intracellular antioxidant systems [5].

Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase that can reduce NO formation by interfering with the L-arginine-structured enzyme of interest [6]. Changes in ADMA availability may also alter NO-established redox signaling and regulate the mobile response to oxidative stress. Nitroglycerin, also known as glyceryl trinitrate, acts as an exogenous NO donor and has been studied as a modifier of tumor response to chemotherapy. Recent experimental evidence indicates that glyceryl trinitrate can regulate ROS-structured pathways and the antitumor interest of doxorubicin in a triple-cancer model may also the experimental device being tested and the biological endpoint [7].

Doxorubicin is widely used in breast cancer treatment and has a cytotoxic play through topoisomerase II inhibition, DNA damage, and changes in cellular redox stability [8]. Redox cycling associated with doxorubicin can produce reactive species, while exposed cells can respond through feeding suppression or increasing additives in their antioxidant defense systems [8]. Total antioxidant capacity provides an inclusive estimate of experimentally testable reduction concerns, however, it does not identify the individual enzymatic and non-enzymatic antioxidants responsible for the measured response [9]. Thus, changes in T-AOC should be interpreted as changes in normal antioxidant capacity rather than direct evidence of cell protection or oxidative damage [9].

The effects of ADMA and nitroglycerin, administered alone or combined with doxorubicin, on antioxidant capacity across breast cancer cell lines with different molecular backgrounds remain insufficiently characterised. The present study therefore evaluated treatment-induced changes in T-AOC-associated absorbance in MCF-7, CAL-51, and MDA-MB-231 breast cancer cell lines, with rat embryonic fibroblast cells included as a non-tumour comparator. The study further examined whether the direction and magnitude of the antioxidant response differed according to the cellular model.

MATERIALS AND METHODS

Study Points, Cell Lines, and Culture Conditions

The present study was modified to be conducted at the Iraq Center for Cancer Medical Genetics Research, Mustansiriyah University, Baghdad, Iraq. Three human breast cancer most mobile lines, MCF-7, CAL-fifty-one, and MDA-MB-231 were obtained

from the cell culture unit of the same center MCF-7 is an estrogen receptor-best breast cancer cell line, while CAL-fifty one MDA-23 fibroblast and MDA-MB-231 breast cancer cell lines, were also obtained from the center and kept as non-tumor fibroblast comparators. The cells were maintained in Roswell Park Memorial Institute (RPMI) medium supplemented with 20% fetal serum (FBS) and incubated at 37°C Culture medium was renewed every 24 h until attached monolayers were established. The cells are finally subcultured using the same old procedure that was used with the help of cell culture unit to maintain cell viability and obtain adequate cultures for experimental treatments.

Experimental design:

After growing of all four types of cells lines and reached to monolayer feature getting twelve falcon of each line and divided as the following:

2Falcon: without any addition or treatments

2Falcon: treated with (10) µmol/mL of ADMA

2Falcon: treated with (200) µmol/mL of Doxorubicin

2Falcon: treated with (10) µmol/mL of ADMA

2Falcon: treated with (40) µmol/mL of nitroglycerin

2Falcon: treated with combination of (10) µmol/mL of ADMA + (200) µmol/mL of Doxorubicin

2Falcon: treated with combination of (40) µmol/mL of nitroglycerine + (200) µmol/mL of Doxorubicin.

To assess the level of total antioxidant and enzymes of antioxidant within the cell lines used Spectrophotometer kits. It primarily serves the purpose of evaluating the total antioxidant capacity of antioxidant solutions. Under acidic conditions, Fe3+-TPTZ is reduced to blue Fe26-TPTZ. The resulting color reaction serves as a trademark for the total antioxidant capacity.

Solution: Zero.Combine nine mL of DW with 20 µL of sulfuric acid (H2SO4) to obtain (forty µmol/mL) FeSO4 solution (well known).

Preparation of solution mixture: (arranged immediately before use) Contains reagent I, reagent II, and reagent III in the ratio 7:1:1, and incubated at 37°C prior to anticipation by the software.

Procedure:

1. Prepare a dilute preferential solution of FeSO4 with concentrations of zero.1, zero.05, 0.1/2, 0.0125, 0.00625 and 0.003125 µmol/mL by means of dilution of forty µmol/mL stock. Then combine a fraction of the standard solution (500 µL) (using DW for pure manipulation) with 500 µL of reagent II. Ensure thorough mixing for a length of 10 min, and finally quantify the absorbance at wavelength of 593 nm.

2. Prior to commencing measurements, preheat the spectrophotometer for a duration of 30 minutes, adjust the wavelength setting to 593 nm, and calibrate the instrument to zero using distilled water.

3. The reagents details as the following:

Reagents	**Blanked tube (AB)**	**Test-tube (AT)**
Solution-mixture	900(µL)	900(µL)
Double DW	120(µL)	90(µL)
Samples	-	30(µL)

Blend the components comprehensively and allow for a duration of ten minutes for the reaction to occur, calibrate the measurement to zero using distilled water, and subsequently ascertain the absorbance at a wavelength of 593 nm.

Statistical Analysis

Data were expressed as representative ± standard deviation (SD) from replicate subculture flasks (n=2 corresponding to each cell line treatment). Statistical analysis was performed using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). In addition to the interaction between cell line and treatment, total antioxidant capacity and cell line measures changed in variance (2 way ANOVA) used to assess main results When large differences are detected, means comparisons were terminated Use least significant difference (LSD) Look after. Differences were considered statistically significant at $P \leq 0.05$ [10].

RESULTS

Total antioxidant capacity-associated absorbance differed according to both the cellular model and the applied treatment (Table 1 and Figure 1). Significant treatment-related differences were detected within REF, MCF-7, CAL-51, and MDA-MB-231 cells at $P \leq 0.05$.

In REF cells, the untreated control recorded a T-AOC-associated absorbance of 0.121±0.02. The lowest value was observed following combined nitroglycerin and doxorubicin treatment (0.055±0.01), which was significantly lower than the untreated control. ADMA (0.077±0.01), doxorubicin (0.104±0.02), ADMA plus doxorubicin (0.061±0.02), and nitroglycerin alone (0.093±0.02) produced intermediate values that did not differ significantly from the control or the nitroglycerin–doxorubicin group according to the shared post-hoc letters.

MCF-7 cells showed a different response pattern. ADMA gave the highest value (0.190±0.03) followed by ADMA plus doxorubicin (0.170±0.03). Both regimens gave significantly greater levels than untreated control (0.115±0.02), doxorubicin only (0.116±0.02) and nitroglycerin plus doxorubicin (0.062±0.02). The lowest value was observed in the combination of nitroglycerin and doxorubicin and it was statistically lower than all other treatments. Nitroglycerin alone gave an intermediate response of 0.162±0.03.

The highest T-AOC-associated absorbance (0.240±0.01) was observed in CAL-51 cells treated with combined nitroglycerin and doxorubicin. This value was significantly higher than the values of untreated cells (0.137 ± 0.03), ADMA treated cells (0.105 ± 0.02), doxorubicin treated cells (0.161 ± 0.04) and nitroglycerin treated cells (0.072 ± 0.02). The combination of ADMA plus doxorubicin produced an intermediate value of 0.179+/-0.01 which was not significantly different from doxorubicin alone or the nitroglycerin-doxorubicin combination. Nitroglycerin alone produced the lowest value which was significantly less than doxorubicin and the two combined treatments.

In MDA-MB-231 cells, doxorubicin alone showed the highest absorbance value correlated with T-AOC (0.277±0.06) and was significantly higher than all other therapy groups. The untreated control, ADMA, ADMA plus doxorubicin, nitroglycerin plus doxorubicin, and nitroglycerin only produced values of 0.151 ± 0.03, 0.135 ± 0.03, 0.119 ± 0.02, 0.137 ± 0.04, and 0.098 ± 0.02, respectively, with no significant differences between these groups.

The T-AOC response was cell-line dependent in terms of both direction and magnitude overall. The nitroglycerin-doxorubicin combination reduced T-AOC-associated absorbance in REF and MCF-7 cells, but significantly increased T-AOC-associated absorbance in CAL-51 cells. The most robust response with Doxorubicin alone was seen in MDA-MB-231 cells, while the ADMA-related increases were most pronounced in MCF-7 cells.

Table 1. Effects of ADMA, Doxorubicin, Nitroglycerin, and Their Combinations on T-AOC-Associated Absorbance in Breast Cancer Cell Lines and REF Cells

Data are presented as mean ± standard deviation from duplicate culture flasks (n=2).

Breast cancer Cell line	**Without treatment**	**Treatments**					LSD value	**P-value**
		ADMA (10) µmol/mL	**Doxo (200) µmol/mL**	**ADMA +Doxo (10+200) µmol/mL**	**NG +Doxo (40+200) µmol/mL**	**NG (40) µmol/mL**		
REF	0.121 ±0.02 A a	0.077 ±0.01 B ab	0.104 ±0.02 B ab	0.061 ±0.02 B ab	0.055 ±0.01 C b	0.093 ±0.02 B ab	0.063 *	0.0470
MCF	0.115 ±0.02 A b	0.190 ±0.03 A a	0.116 ±0.02 B b	0.170 ±0.03 A a	0.062 ±0.02 B c	0.162 ±0.03 A ab	0.071 *	0.0497
CAL	0.137 ±0.03 A bc	0.105 ±0.02 B bc	0.161 ±0.04 B b	0.179 ±0.01 A ab	0.240 ±0.01 A a	0.072 ±0.02 B c	0.075 *	0.0441

MDA	0.151 ±0.03 A b	0.135 ±0.03 AB b	0.277 ±0.06 A a	0.119 ±0.02 AB b	0.137 ±0.04 B b	0.098 ±0.02 B b	0.072 *	0.0376
LSD value	0.044 NS	0.067 *	0.062 *	0.071 *	0.074 *	0.068 *	---	---
P-value	0.5028	0.0487	0.0466	0.0326	0.0291	0.0489	---	---

Different lowercase letters within the same row indicate significant differences among treatments, whereas different uppercase letters within the same column indicate significant differences among cell models. Means sharing at least one letter are not significantly different. Statistical significance was defined as P≤0.05. ADMA, asymmetric dimethylarginine; Doxo, doxorubicin; NG, nitroglycerin; REF, rat embryonic fibroblasts.

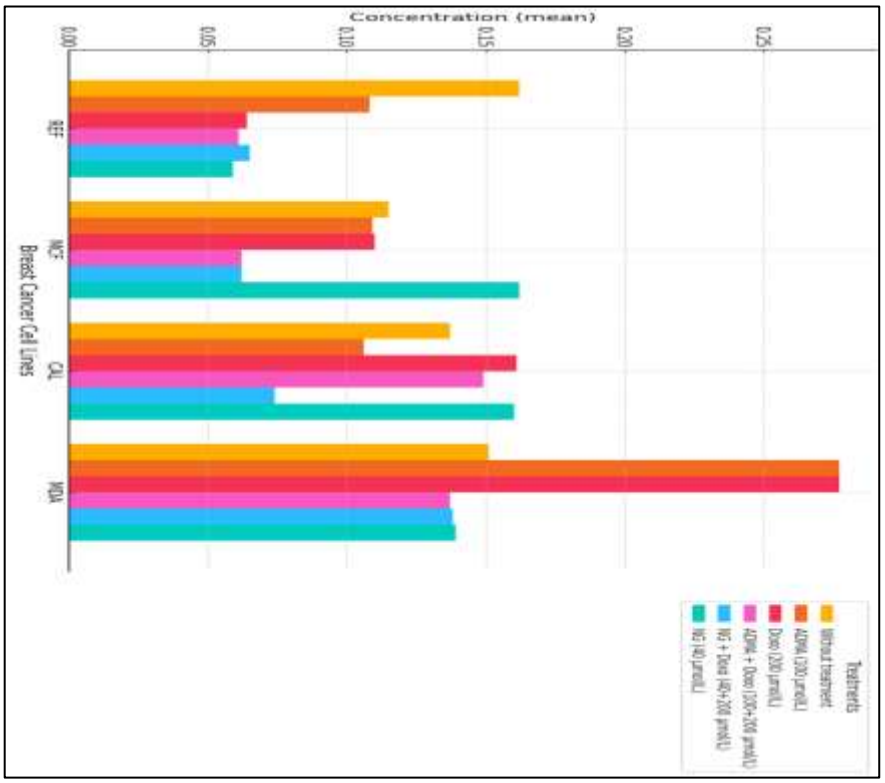

Figure 1. Treatment-Induced Changes in T-AOC-Associated Absorbance Across the Examined Cell Models

Discussion

A look at the donation revealed that ADMA, nitroglycerin, doxorubicin, and their mixtures produced abnormal T-AOC-associated uptake responses in all REF, MCF-7, CAL-51, and MDA-MB-231 cells (Table 1). This variant is stabilized by the prominent molecular and functional heterogeneity among mobile strains of most breast cancers, which may also affect their response to redox-modulatory measures [3]. As a result, the results of the studied compounds should be uniformly classified as antioxidants or spice oxidants and preferably interpreted in accordance with the cellular model [3].

In REF cells, combined nitroglycerin and doxorubicin produced the lowest T-AOC-related uptake, whereas the other treatments produced intermediate values, not all of which were markedly different from untreated manipulations (Table 1) Fe3+-TPTZ assay quantitatively measures intracytoplasmic reduction specificity [11]. In addition, the total antioxidant capacity does not identify the characteristic enzymatic or non-enzymatic additives responsible for the measured signal [9]. The REF response should be interpreted as a decrease in assay-detectable antioxidant capacity as a result, with recruitment of the specific oxidative mechanism out [9].

A typical sample of MCF-7 cells was demonstrated because ADMA by itself and ADMA mixed with doxorubicin rather exaggerated T-AOC-related uptake, whereas nitroglycerin mixed with doxorubicin produced the least opposition (Table 1) [6], Therefore, the extended response after ADMA treatment may also reflect a dynamic adaptation of altered nitric oxide priority regulation as opposed to the direct antioxidant effect of ADMA [6]. Because the T-AOC assays are induced through their reaction chemistry, the improved costs cannot be mechanistically interpreted as evidence of reduced oxidative damage or improved cell survival [12]. The lack of measurements of intracellular reactive oxygen species, glutathione, antioxidant enzymes, and cell viability prevents the identification of the mechanism underlying the MCF-7 response [9].

CAL-fifty-one cells showed very good T-AOC uptake after the combined treatment of nitroglycerin and doxorubicin, while nitroglycerin by itself produced minimal charge (Table 1). This response was in contrast to that determined in REF and MCF-7 cells after the same mixture treatment (Table 1). The increased charge may suggest an expanded production of assay-reactive degradation material or even a compensatory response to treatment-related stress, but it does not indicate protection against oxidative damage or treatment resistance [9]. The differences between antioxidant assays additionally indicate that changes in reducing capacity cannot be equated with total intracellular redox state at once [12].

In MDA-MB-231 cells, doxorubicin produced the highest T-AOC-related uptake while contrast treatments remained vs. untreated control (Table 1 breast cancer cells have also shown that doxorubicin can alter mitochondrial homeostasis, power metabolism, and redox regulation in a cell line-dependent manner [13]. Therefore, the multiplied T-AOC-related uptake may additionally create a favorable increase in decreasing capacity, although current experiments cannot determine whether this reflects differences in antioxidant defense, oxidative damage, altered metabolism, or viable cell volume [9].

The response to nitroglycerin depended on the cell line and its mixture with doxorubicin (Table 1). A current study suggests expanded intracellular nitric oxide and activation of apoptosis-related pathways using a NO donor spinoff in MCF-7 cells, indicating that NO delivery can affect strategies not represented by T-AOC now itself [14]. A photoactivated doxorubicin-NO-releasing hybrid additionally reduced the viability of breast cancer cells and caused drug accumulation, although this treatment tool is different from the separate administration of nitroglycerin and doxorubicin used

within the present [15]. Without further delay, glyceryl trinitrate improved the antitumor results of doxorubicin in the 4T1 triple-poor breast cancer strain and altered the ROS-dependent immunosuppression pathways [7]. On the other hand, that study used tumor-bearing animals, immunocytosis, and cell loss of survival endpoints, while the dominant study measured uptake of T-AOC in single cell cultures [7] Modern effects thus between nitroglycerin and doxorubicin cytocorbicity and viability tumor growth was not evaluated [7].

The contrasting responses of CAL-51 and MDA-MB-231 cells are relevant because each is classified as a triple-malignant breast-most cancer model but displayed distinct T-AOC styles (Table 1) [3], [4]. Therefore, receptor classification by itself is not sufficient to predict antioxidant capacity responses to ADMA, nitroglycerin, or doxorubicin [3]. The combined measures, moreover, elicited responses that could not be expected from the corresponding individual measures (Table 1). However, the study did not include dose metrics or formal drug interaction analysis, and as a result, the findings should be described as cell line-dependent mixed results preferably for evidence of pharmacological synergy (Materials and Methods).

Study Limitations

The study was limited by its in vitro design, the use of a single concentration for each compound, duplicate culture flasks (n=2), and reliance on T-AOC as the only redox-related measurement. The absence of complementary assessments of intracellular reactive oxygen species, cell viability, apoptosis, and antioxidant enzymes limits the mechanistic interpretation of the findings.

CONCLUSION

Fundamental Finding: ADMA, nitroglycerin, and doxorubicin induced cell line-dependent changes in the uptake of T-AOC. Contradictory responses among MCF-7, CAL-fifty one, MDA-MB-231, and REF cells indicate that antioxidant modulation depends on mobile historical past. **Implication:** These findings indicate that antioxidant responses to ADMA, nitroglycerin, and doxorubicin vary according to the characteristics of the cell line. **Limitation:** The study does not explicitly report complementary oxidative stress, cell viability, or apoptosis assays to validate the observed T-AOC responses. **Future Research:** These results need to be confirmed using complementary oxidative pressure, cell viability, and apoptosis assay.

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