

Effects of Vitamin E on Doxorubicin Cytotoxicity in Human Breast Cancer Cells *in Vitro*

Mohadeseh Ahmadi¹, Akbar Hedayatizadeh-Omran^{1*}, Reza Alizadeh-Navaei¹, Majid Saedi², Ehsan Zaboli¹, Omolbanin Amjadi¹, Hamidreza Kelidari², Zahra Besharat¹

Abstract

Objective: This study aimed to evaluate in vitro synergistic anticancer effect of doxorubicin combined with Vitamin E. **Methods:** The MTT assay was utilized to assess the cytotoxicity of Vitamin E and vitamin E combined with doxorubicin and vital activities of SKBR3, MDA-MB-231, and HFF cells over a 24-hour incubation period. In addition, the antioxidant properties of these interventions and the decrease of reactive oxygen species (ROS) content caused by the treatment were evaluated. **Results:** The antiproliferative effect of doxorubicin increased significantly in combination with vitamin E (Doxcorobicin 2 μ M vs. Vitamin E 120 μ M, P=0.000). Despite reducing cell ROS content due to vitamin E treatment, the combination of vitamin E and doxorubicin showed no significant synergistic effect (Doxcorobicin 2 μ M vs. Vitamin E 120 μ M, P=0.998). **Conclusion:** This study indicated that the doxorubicin–vitamin E treatment reduced the viability of breast cancer cells with the minimum side effects on normal cells. In addition, the high dosage of vitamin E intensified the cytotoxicity of doxorubicin.

Keywords: Antioxidant- breast cancer- doxorubicin- vitamin E

Asian Pac J Cancer Prev, **23** (1), 201-205

Introduction

Breast cancer is a leading cause of cancer death among females worldwide in the world. To fight against breast cancer, developing therapeutic strategies is essential. Due to irreversible tissue toxicity, the clinical use of doxorubicin (DOX) as a broadspectrum anti-breast cancer agent has been restricted. A combination of therapeutic interventions can provide patients with the opportunity to make the most of treatment (Samare-Najaf et al., 2020; Wei et al., 2020).

In biological systems, vitamin E is the most critical lipophilic antioxidant to form the frontline defense against the peroxidation of unsaturated fatty acids (Sylvester, 2007). Recently, the role of this vitamin has been identified in reducing the growth of cancerous cells. It has also been proven that vitamin E can act as an antioxidant agent to inhibit estrogen receptor-positive breast tumors (Larouche et al., 2017). Some studies showed that the serum levels of vitamin E below 4.7 μ g/ml had a significant correlation with breast cancer. Specific types of vitamin E showed potential apoptotic activities against a wide range of tumor cells. Still, they had no such effects on the functions and survivability of normal cells (Dorjgochoo et al., 2009). Since the use of DOX in breast cancer treatment is

associated with high side effects and the effect of vitamin E on the survival of cancer cells has fewer side effects, the combination of these interventions was evaluated. This strategy may provide new insight into increasing the efficacy of DOX due to its association with vitamin E and reducing the side effects of DOX.

Materials and Methods

Material

Vitamin E (Vit E), were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Doxorubicin (DOX) was purchased from (Ebeve. pharma, Tehran, Iran). The breast cancer cell lines of MDA-MB-231 (NC: C578) and the normal cell lines of HFF (NC: 452) were purchased in frozen vials from the Pasteur Institute of Iran. SKBR3 (NCBI code: C170) was kindly obtained from Dr. Valadan (Department of Immunology, Molecular and Cell Biology Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran).

Cell Culture

The breast cancer cell lines of SKBR3, MDA-MB-231, and the normal cell lines of HFF were cultured in

¹Gastrointestinal Cancer Research Center, Non-Communicable Disease Institute, Mazandaran University of Medical Sciences, Sari, Iran. ²Department of Pharmaceutics, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.
*For Correspondence: akbar_hedayati@yahoo.com

RPMI-1640 with 10% of the fetal Bovin serum (FBS), 100 U/ml of penicillin, and 100 µg/ml of streptomycin. The cell flask was placed in an incubator at 37°C, 5%-CO₂ pressure, and 95% humidity. Dosage and time were evaluated to determine the optimal effects of vitamin E. The cells were then treated with 0-µM, 30-µM, 60-µM, and 120-µM concentrations of vitamin E, 2-µM and 4-µM concentrations of doxorubicin, and the doxorubicin–vitamin E mixture. They were then analyzed after a 24-hour incubation period. The zero concentration of medicine was considered the control group, and the experiments were triplicated to increase the accuracy of comparison and process efficiency.

Cytotoxic Assay of Vitamin E

The MTT assay was conducted to analyze the cytotoxicity of synthesized vitamin E on the cell lines of interest. The MTT powder is the water-soluble yellow tetrazolium salt retrieved by the succinate dehydrogenase enzyme of the mitochondria of living cells and then converted into the water-insoluble formazan dyes. In brief, 15×10³ of MDA-MB-231 and SKBR3 cells were cultivated in a 96-well plate. The cells were treated with the designated interventions in the new environment overnight for 24 hours, after which the MTT solution (5 mg/ml in PBS) was added to the plate wells. The plate was then placed in an incubator for 3 hours. The superficial solution was then extracted, and 200 µl of dimethyl sulfoxide (DMSO) was added to each well to tear the cell membrane and let formazans out. The plate was placed on a shaker for 20 minutes. Finally, the specimens were measured with a microplate reader at a wavelength of 540 nm and a frequency of 660.

The results of treating SKBR3, MDA-MB-231, and HFF cells with the designated concentrations of vitamin E, doxorubicin, and a combination of these interventions were plotted on diagrams for 24 hours. The IC₅₀ of the medicine (i.e., a concentration that inhibits 50% of cell growth) was calculated for cancer cell lines in a web-based application developed by AAT Bioquest Inc.

Intracellular reaction oxygen species scavenging activity

The cellular reactive oxygen species (ROS) content was measured through a ROS-sensitive fluorescence indicator called DCFH-DA (Abcam, US. Cat No.: AB113851). Their scavenging activities were evaluated through vitamin E, doxorubicin, and a combination of these two interventions. For this purpose, 15×10³ of MDA-MB-231 cells were cultivated in each well of a 96-well plate. After 24 hours, they were then incubated in the presence of 60-µM and 120-µM concentrations of vitamin E, 2-µM and 4-µM concentrations of doxorubicin, and a combination of these interventions without changing the culture environment for 20 hours at 37° C with 5% of CO₂. After the wells were washed with a buffer, the cells were incubated with DCFH-DA (25 µM) for 45 minutes in a medium free of any red phenol at 37°C. The fluorescent intensity was measured by a microplate reader (Bio Tech-USA) at 485/535 wavelengths.

Statistical Analysis

Data analysis was performed in SPSS 21. After the normality of variable distribution was checked, ANOVA was employed to evaluate the mean of cellular antioxidant proliferation. The significance level was considered P<0.05, whereas IC₅₀ was calculated for different cell lines in a web-based application developed by AAT Bioquest Inc.

Results

Results of Analyzing Cytotoxicity of Vitamin E

The measured cytotoxicity was analyzed in MDA-MB-231, SKBR3, and HFF cells based on the vitamin E treatment with different doses. Generally, there were significant differences in cytotoxicity rates caused by different treatments of Vitamin E in cancerous cells (p=0.000), whereas no significant difference was observed in the normal HFF cells (p=0.658) (Figure 1). On the other hand, there were significant differences

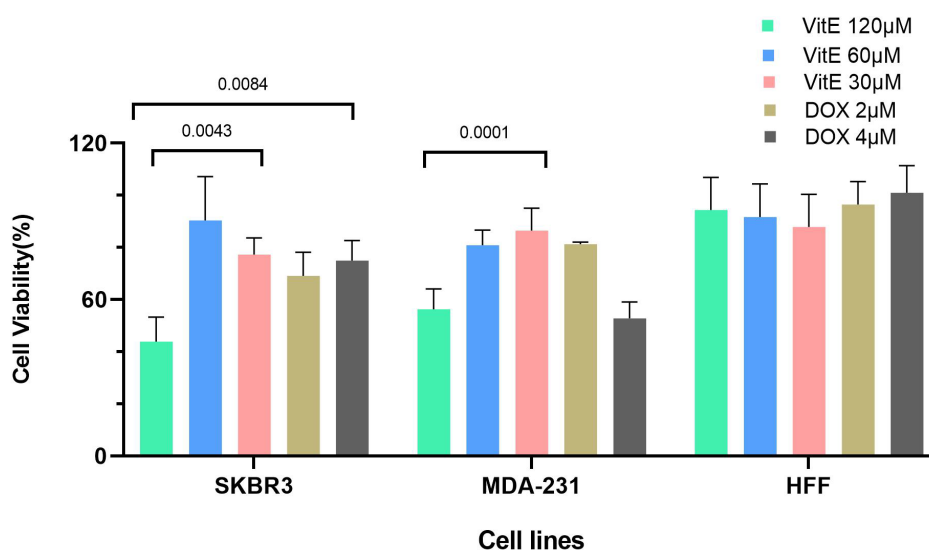


Figure 1. Comparing SKBR3, MDA-MA-231, and HFF in Survivability in the Presence of Vitamin E and Doxorubicin Over 24 hours. Data are presented as mean ± standard deviation. All data are depicted as a percentage with respect to the control.

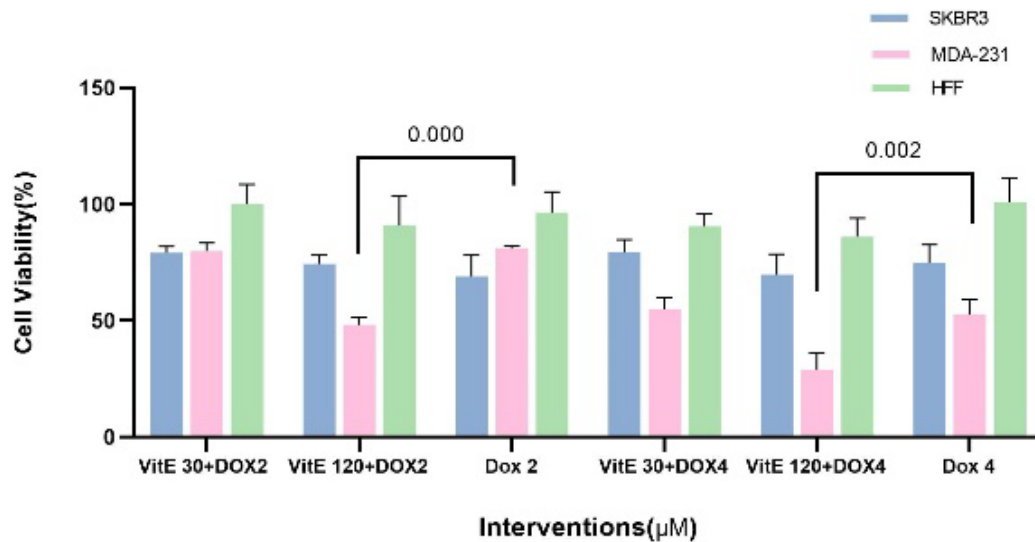


Figure 2. Comparing SKBR3, MDA-MA-231, and HFF in Survivability in the Presence of Doxorubicin and the Combination of Vitamin E and Doxorubicin Over 24 Hours. Data are presented as mean \pm standard deviation. All data are depicted as a percentage with respect to the control.

between SKBR3, MDA-231, and HFF ($p=0.0001$) in terms of toxicity after treatments with vitamin E (120 μM), doxorubicin (4 μM), and the combination of these interventions (Vit E 120 + DOX 4). These results show the considerable cytotoxicity of the combination of interventions on cancerous cells with lower toxic effects on normal cells. There was no significant difference between the cytotoxicity induced with vitamin E (120 μM) and doxorubicin (4 μM) in MDA-231 cells ($p=0.823$). In contrast, the combination of these interventions indicated significant cytotoxicity compared with doxorubicin per se ($p=0.0236$). According to Figure 2, vitamin E had significant effects on the efficiency of doxorubicin in the combination of interventions (Vit E 120 + DOX 2, $p=0.000$) (Vit E 120 + DOX 4, $p=0.002$). In other words, the cytotoxicity of Vit E + DOX was significantly higher than that of only doxorubicin on MDA-MA-231 cells

($p=0.000$) (Figure 2). The IC_{50} values of vitamin E on SKBR3 and MDA-231 cell lines in 24 hours were 119 μM and 151 μM , respectively.

Antioxidant Activity of Vitamin E

The intracellular ROS was measured to determine the antioxidant effects of vitamin E per se and in combination with doxorubicin. The ROS generation decreased in the 24-hour treatment of MDA-MA-231 cells with vitamin E (120 μM), doxorubicin (4 μM), and the combination of these interventions (Vit E 120 + DOX 4) (Figure-4). The reduction of peroxide ions was identified through the evidence for the attraction of ROS-sensitive fluorescence indicators called DCFDH. In addition, the cells treated with vitamin E (120 μM) had significantly lower fluorescence than the untreated cells ($p=0.019$). Despite reducing ROS content in the combination group (Vit E

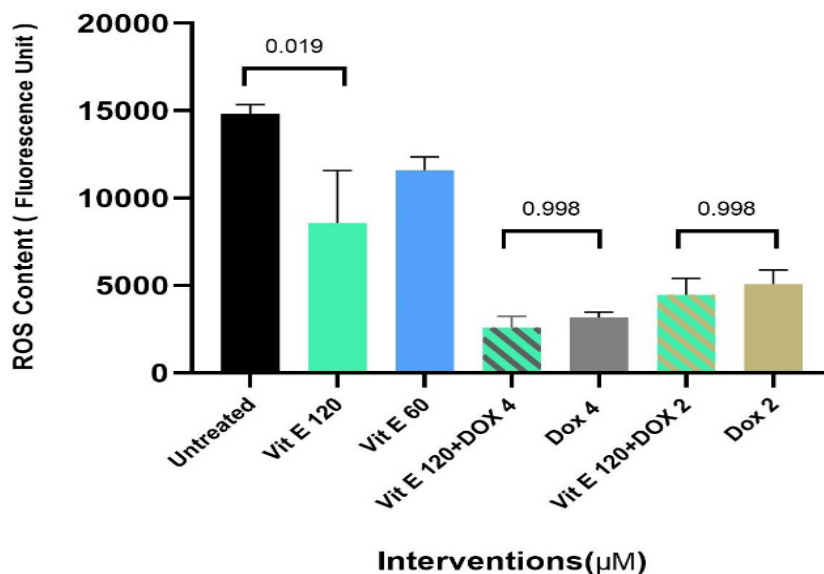


Figure 3. Measuring the Antioxidant Activity of Vitamin E in the Presence of Oxidative Stress in MDA-MA-231 through DFCH-DA Fluorescence.

120 + DOX 4; Vit E 120 + DOX 2) as opposed to DOX 4 and DOX 2, the difference was not significant ($p=0.998$).

Discussion

The effective treatment of breast cancer requires maximum therapeutic effectiveness with minimum inappropriate effects. A combination of therapeutic interventions can provide patients with the opportunity to make the most of therapy. This approach can guarantee a good quality of life for patients and reduce or stop relapse and decrease therapeutic resistance or cytotoxicity effects of selective treatments (Fisusi and Akala, 2019). This is the first study to analyze the effectiveness of a combination of vitamin E and doxorubicin against the proliferation of breast cancer cells. In this study, the cell culture model was selected to experiment with the laboratory effects of different doses of vitamin E with doxorubicin (Vit E + DOX) on the proliferation of MDA-MB-231 SKBR3 and HFF, and antioxidant effect in a controlled empirical environment. Vitamin E is an antioxidant nutrient having a wide range of dosages for clinical purposes. Generally, 5–80- μ M vitamin E is used for the mixed clinical treatments of different diseases (Wei et al., 2019). In this study, the maximum dose was more than this range. According to Figure 1, a 120- μ M amount of vitamin E caused higher cytotoxicity than an 80- μ M dose in cancerous cells; however, it caused lower cytotoxicity effects on normal cell lines which simultaneous examination of normal and cancer cells has been one of the strengths of the above study. Ongoing proliferation is the prominent characteristic of cancerous cells, whereas the MTT assay is the most common method of cellular proliferation. In this study, the antiproliferative effect of doxorubicin increased significantly in combination with a 120- μ M dose of vitamin E. Yiang et al. reported the reduced survivability of renal fibroblast cells (NRK-49F) as a dosage-dependent reaction caused by vitamin E (Yiang et al., 2016). This finding is consistent with the results of this study. According to Yiang et al., a derivative of vitamin E (α -Tocopherol) increased the antiproliferative effects of gefitinib in cells.

Moreover, the results of previous studies (Wei et al., 2019) showed that treatment with α -Tocopherol (a derivative of vitamin E) with methotrexate had synergistic antiproliferative effects and decreased the proliferation of MDA-MB-231. Furthermore, combining vitamin E with statins had synergistic antiproliferative and proapoptotic effects on various cancerous cells, including the intestine and breast (Jiang, 2019). Accordingly, vitamin E is an effective antiproliferative agent for cancerous cells in laboratory conditions. The results of this study are consistent with the reported findings. However, Peralta and Uchihara showed that α -Tocopherol might decrease some chemotherapy medicines such as tamoxifen and crizotinib (Peralta et al., 2006; Uchihara et al., 2017). As the side product of metabolism, ROS plays a crucial role in maintaining hemostasis. In addition to the enzymes produced by cells to reduce the ROS-caused loss, a few small molecules such as vitamin E can act as cellular

antioxidants to inhibit tumor growth. Previous studies showed that vitamin E had antioxidant functions in chemotherapy and reduced the side effects of chemical agents (Peh et al., 2016; Yüncü et al., 2015). In this study, the antioxidant effect of vitamin E per se was greater than that of doxorubicin; however, no synergistic effects were observed in the combination of vitamin E and doxorubicin. Zal et al., (2018) confirmed the protective role of vitamin E in combination with folate. According to Zal et al., (2018), endometrial cells receiving oxidative stress from the treatment with vitamin E experienced longer survivability than untreated cells. In addition, Diao confirmed that vitamin E would reduce ROS generation in the tumor tissue and MCF7 cells but improve tumor growth (Diao et al., 2016). Finally, this study indicated that mixed treatment with doxorubicin and vitamin E reduced the survivability of breast cancer cells with the minimum side effects on normal cells. In addition, high doses of vitamin E increased the cytotoxicity effects of doxorubicin.

Author Contribution Statement

Conceptualization, M.A., A.H.O., and R.A.N.; methodology, M.A., A.H.O., O.A, and R.A.N; software, M.A., Z.B. and R.A.N.; validation, M.S. and H.K.; formal analysis, M.A.; investigation, R.A.N., A.H.O. and E.Z; writing—original draft preparation: M.A.; writing—review and editing, A.H.O., R.A.N. and M.S.; visualization, M.A, project administration, A.H.O. All authors have read and agreed to the published version of the manuscript.

Acknowledgements

Authors would like to thank the staff of the Non-communicable disease institute. This study is a part of thesis in the fulfillment of the requirements for the degree of Doctor of Medicine.

Funding statement

This research was received support from deputy of research and technology of Mazandaran University of Medical Science (grant No.6851).

Ethical approval

This study was approved by the Research Ethics Committees of Mazandaran University of Medical Sciences (IR.MAZUMS.IMAMHOSPITAL.REC.1398.155).

Availability of data

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declaration of conflict of interest

All the authors declare no conflict of interest.

References

Diao Q, Zhang J, Zhao T, et al (2016). Vitamin E promotes breast

- cancer cell proliferation by reducing ROS production and p53 expression. *Eur Rev Med Pharmacol Sci*, **20**, 2710-7.
- Dorjgochoo T, Gao Y-T, Chow W-H, et al (2009). Plasma carotenoids, tocopherols, retinol and breast cancer risk: results from the Shanghai Women Health Study (SWHS). *Breast Cancer Res Treat*, **117**, 381-9.
- Fisusi FA, Akala EO (2019). Drug combinations in breast cancer therapy. *Pharm Nanotechnol*, **7**, 3-23.
- Jiang Q (2019). Natural forms of vitamin E and metabolites-regulation of cancer cell death and underlying mechanisms. *IUBMB Life*, **71**, 495-506.
- Larouche D, Hanna M, Chang S-L, et al (2017). Evaluation of antioxidant intakes in relation to inflammatory markers expression within the normal breast tissue of breast cancer patients. *Integrat Cancer Ther*, **16**, 485-95.
- Peh HY, Tan WD, Liao W, et al (2016). Vitamin E therapy beyond cancer: Tocopherol versus tocotrienol. *Pharmacol Ther*, **162**, 152-69.
- Peralta EA, Viegas ML, Louis S, et al (2006). Effect of vitamin E on tamoxifen-treated breast cancer cells. *Surgery*, **140**, 607-15.
- Samare-Najaf M, Zal F, Safari S, et al (2020). Stereological and histopathological evaluation of doxorubicin-induced toxicity in female rats' ovary and uterus and palliative effects of quercetin and vitamin E. *Hum Exp Toxicol*, **39**, 1710-24.
- Sylvester PW (2007). Vitamin E and apoptosis. *Vitamins Hormones*, **76**, 329-56.
- Uchihara Y, Ueda F, Tago K, et al (2017). Alpha-tocopherol attenuates the anti-tumor activity of crizotinib against cells transformed by NPM-ALK. *PLoS One*, **12**, e0183003.
- Wei CW, Yu YL, Chen YH, et al (2019). Anticancer effects of methotrexate in combination with α -tocopherol and α -tocopherol succinate on triple-negative breast cancer. *Oncol Rep*, **41**, 2060-6.
- Wei T, Xiaojun X, Peilong C (2020). Magnoflorine improves sensitivity to doxorubicin (DOX) of breast cancer cells via inducing apoptosis and autophagy through AKT/mTOR and p38 signaling pathways. *Biomed Pharm*, **121**, 109139.
- Yiang GT, Chen JN, Lin PS, et al (2016). Combined treatment with vitamin E and gefitinib has synergistic effects to inhibit TGF- β 1-induced renal fibroblast proliferation. *Mol Med Rep*, **13**, 5372-8.
- Yüncü M, Bükücü N, Bayat N, et al (2015). The effect of vitamin E and L-carnitine against methotrexate-induced injury in rat testis. *Turk J Med Sci*, **45**, 517-25.
- Zal F, Khademi F, Taheri R, et al (2018). Antioxidant ameliorating effects against H₂O₂-induced cytotoxicity in primary endometrial cells. *Toxicol Mech Methods*, **28**, 122-9.



This work is licensed under a Creative Commons Attribution-Non Commercial 4.0 International License.