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ASCORBYL PALMITATE ENHANCES ANTI-PROLIFERATIVE EFFECT OF TRASTUZUMAB IN HER2-POSITIVE BREAST CANCER CELLS

Background. Breast cancer (BC) accounts for about 30% of cancers in women, with a mortality rate of around 15%. HER2-positive BC, an aggressive subtype, represents 15—20% of all BC cases. High-dose vitamin C has shown antitumor effects by increasing reactive oxygen species in cancer cells without significant toxicity. **Aim.** This study aimed to explore an in vitro dual treatment using ascorbyl palmitate (AP), a lipophilic vitamin C derivative, and trastuzumab, an HER2 receptor blocker. **Materials and Methods.** HER2-positive SK-BR-3 BC cells were treated with AP, trastuzumab, or their combination. The cell survival MTT assay, apoptosis, and cell cycle phase analysis were conducted using flow cytometry, while mRNA and protein expression were assessed using RT-qPCR and Western blot methods. Ki-67 expression was evaluated by immunofluorescence assay. **Results.** AP reduced cell viability in a time- and dose-dependent manner, and its combination with trastuzumab further decreased cell viability. A cytometric analysis showed enhanced apoptosis after combination treatment. mRNA analysis revealed upregulated *TP53* mRNA expression, along with upregulation of *BAX, CYCS, CASP3,* and *CASP8* gene expression, while the *BCL-2* and *BCL2L1* genes were downregulated, further supporting the induction of apoptosis. The antiproliferative effectiveness of the combination therapy was demonstrated by a Western blot assay, which showed suppression of phospho-P38, ERK1/2, and PI3K protein synthesis. **Conclusion.** These results underscore the potential effects of combining AP and trastuzumab in BC treatment, guiding future therapeutic strategies.

Keywords: breast cancer, trastuzumab, HER2-positive, ascorbyl palmitate, apoptosis, ascorbic acid palmitate.

Breast cancer (BC) is one of the leading causes of morbidity and mortality among women, according to the World Health Organization data [1]. BC exhibits heterogeneous morphological and biological diversity, necessitating varied treatment protocols [2]. HER2-positive BC is one of the five main subtypes of BC, characterized by the overexpression of the human epidermal growth factor receptor 2 (HER2) protein, which promotes cancer cell growth and division [3]. BC treatment involves a multidisciplinary approach, in which therapeutic options such as radiotherapy, surgery, chemotherapy, and immunotherapy can be combined in different sequences. Effective treatment of BC requires maximizing therapeutic efficacy with minimal adverse effects to ensure good quality of life for patients. Pa-

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tients with early-stage BC undergo breast-conserving surgery with radiotherapy or mastectomy. Adjuvant chemotherapy, biological therapy, and endocrine therapy are used in most women based on proven survival benefits and individualizing risk-based therapy, which is now a clinical reality for patients with hormone receptor-positive cancer [4].

Patients with HER2-positive BC receive combination therapy with chemotherapy and HER2-targeted treatment. The presence of HER2-targeted agents significantly alters the prognosis of HER2-positive BC patients. Initial trials randomizing patients to trastuzumab (Herceptin), a monoclonal antibody targeting the HER2 receptor, or chemotherapy alone demonstrated a reduction in recurrence rates by approximately 50% [5, 6]. Overexpression of the HER2 receptor is observed in approximately 20—30% of BC patients and is associated with poor prognosis [7, 8]. Trastuzumab inhibits cell proliferation and survival-associated signaling pathways by blocking HER2 [6]. While some metastatic patients respond to trastuzumab as a single agent, approximately 60% of patients develop resistance after the initial response [9]. Currently, there is no single standard treatment for most HER2-positive BC patients, hence there is a need for new treatment modalities. Receptor tyrosine kinases (RTKs) regulate critical biological processes including cell proliferation, differentiation, metabolism, and survival by activating a series of intracellular signaling cascades [10]. Abnormal expression and mutations in genes encoding RTKs are well known to contribute to the development of various diseases, including cancer. Upon stimulation, phosphorylated amino acids regulate numerous downstream signaling pathways and proteins, including PI3K/AKT, Ras/MEK/ERK, PLCγ/protein kinase C, and JAK/STAT [11—13]. Members of the ERBB family, such as HER2 and EGFR, are frequently overexpressed in various cancers, including BC, and are targets of many FDA-approved drugs like tyrosine kinase inhibitors (TKIs) and monoclonal antibodies (mAbs) targeting their ectodomains [14].

Vitamin C, also known as ascorbate or L-ascorbic acid, is an essential water-soluble vitamin required for biosynthesis of carnitine, collagen, and neurotransmitters. High doses of ascorbic acid, used as a pharmacological agent, have demonstrated anticancer activity in various cell lines, including breast, ovarian, pancreatic, and hepatocellular ones [15, 16]. Ascorbyl palmitate (AP), an ester formed from ascorbic acid and palmitic acid that creates a fat-soluble form of vitamin C, has garnered significant interest due to its lipophilic properties, which allow it to readily penetrate cell membranes and cross the blood-brain barrier [17]. The synonyms of AP include L-ascorbyl palmitate, palmitoyl L-ascorbic acid, ascorbic acid palmitate, ascorbyl palmitic acid, vitamin C palmitate, 6-O-palmitoyl-L-ascorbic acid, ascorbyl-6-palmitate, and palmitoyl ascorbate. The combination of high-dose vitamin C with trastuzumab effectively inhibits BC cell proliferation and migration by reducing oxidative stress in the tumor microenvironment and potentially enhancing the immune response against cancer cells [18]. Additionally, AP significantly reduces mammary tumors in mice, with its micellar particles demonstrating even greater efficacy [19]. AP is proposed to exert cytotoxic effects through several mechanisms, including cell cycle arrest, induction of apoptosis, and inhibition of proliferation. Furthermore, its hydrophobic properties may increase cell membrane permeability, which could further contribute to cell death [20, 21]. Additionally, AP has been reported to synergistically interact with certain chemotherapeutic agents; specifically, combinations with drugs like docetaxel or paclitaxel have shown effectiveness in some cancer models [15, 22]. For example, AP demonstrates strong cytotoxic effects at a concentration of 10.1 μg/mL in leukemia cells [23] and suppresses proliferation at 350 μM in the human oral cancer cell lines [24].

As there has not yet been research on combination therapy with AP and a monoclonal antibody like trastuzumab, this study was planned considering the advantages of its biological use compared to vitamin C.

Materials and Methods

Cell culture. Human SK-BR-3 BC cells were obtained from Dr. Yelda Birinci Kudu at the Istinye University in Türkiye. These cells were grown in a 50/50 mixture of Dulbecco's modified Eagle medium (DMEM) and Ham's F12 (Wisent, Canada), supplemented with 2 mM L-glutamine, 1,500 mg/L sodium bicarbonate, and 10% fetal bovine serum (Life Technologies, USA). The cells were cultured at 37 °C in an atmosphere of 5% CO_2.

Cell survival assay. SK-BR-3 cells (1×10^4 cells) were seeded in 96-well plates (Sunub, China) and incubated for 24 h. Subsequently, the medium was replaced with fresh medium containing various concentrations of AP (ChemCruz Biochemicals, Nederland) (7.8 μM–1000 μM) or 3.125 μg/mL–800 μg/mL trastuzumab (Genentech, USA) and incubated for 72 h. After determining the doses that resulted in approximately 50% cell death, 100 μM AP, 400 μg/mL trastuzumab, or their combination were administered for 72 h. Cell survival was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) test (Across Organics, USA), dissolving the resulting formazan crystals with DMSO and measuring absorbance at 570 nm using a plate reader (Multiscan GO, Thermo Scientific, Finland). The viability was calculated as OD of the sample/OD of the blank control × 100 [25].

Assessment of cell cycle progression. The cells were treated for 72 h with 100 μM AP, 400 μg/ml trastuzumab, or their combination. Subsequently, SK-BR-3 cells were detached with trypsin, fixed in 70% ethanol, and stored overnight at –20 °C. The cells were then washed with cold PBS, and cell cycle analysis was conducted using a commercial kit (Life Technologies, USA). The distribution of cell-phase populations (G1, S, and G2/M) was determined using a Tali image-based cytometer (Invitrogen/Life Technologies, USA) following the manufacturer's instructions [26].

Primer sequences used in this study

Gene	Primer sequences (5′-3′)
BAX	F: CATGGGCTGGACATTGGACT R: AAAGTAGGAGAGGAGGCCGT
BCL2	F: TTGTGGCCTTCTTTGAGTTCGGTG R: GGTGCCGGTTCAGGTACTCAGTCA
BCL2L1 (Bcl-xL)	F: GTAAACTGGGGTCGCATTGT R: TGGATCCAAGGCTCTAGGTG
CASP3 (Caspase-3)	F: TGGTTCATCCAGTCGCTTTG R: CATTCTGTTGCCACCTTTCG
CASP8 (Caspase-8)	F: CTGCTGGGGATGGCCACTGTG R: TCGCCTCGAGGACATCGCTCTC
CYCS (Cytochrome c)	F: TGGGCCAAATCTCCATGGTC R: AGGCAGTGGCCAATTATTACTCA
TP53	F: GAGGTTGGCTCTGACTGTACC R: TCCGTCCCAGTAGATTACCAC
CDKN1A (p21)	F: GGCGTTTGGAGTGGTAGAAA R: GACTCTCAGGGTCGAAAACG
CDKN1B (p27)	F: TTGCAGGTCGCTTCCTTATT R: TGGGCCAAATCTCCATGGTC
GAPDH	F: TTGGTATCGTGGAAGGACTCA R: TGTCATCATATTTGGCAGGTTT

Determination of apoptotic cells. SK-BR-3 cells were seeded overnight at a concentration of 1×10^6 cells per 25 cm² flasks (Nest, China). Following the overnight incubation, the cells were maintained in 100 μM AP, 400 μg/ml trastuzumab, or their combination for 72 h. The cells were then trypsinized, rinsed with PBS, resuspended in annexin V binding buffer (ABB), washed again, resuspended, and stained with Annexin V/propidium iodide (PI) (Invitrogen, USA). The stained cells were then left to incubate for 15 min at room temperature in the dark. The samples were centrifuged at 300 g for 5 min, resuspended in ABB, and reincubated with a PI solution in the dark. An image-based cytometer (Invitrogen, Life Technologies, USA) was used to determine the proportions of total and apoptotic cells [26].

Evaluation of mRNA expression. Cells were treated with 100 μM AP, 400 μg/ml trastuzumab, or their combination for 72 h, followed by the isolation of total RNA. The RNA samples were then converted into complementary DNA using a cDNA synthesis kit from Thermo Fisher Scientific (USA). mRNA expression levels were measured using an RT-qPCR system (Applied Biosystems, USA) with SYBR Green PCR Master Mix (Thermo Fisher Scientific, USA). GAPDH served as an internal control. The oligonucleotide primers, detailed in the Table, were synthesized by PRZ Biotech (Ankara, Türkiye).

Immunoblot analysis. Proteins were extracted from SK-BR-3 cells treated for 72 h with 100 μM AP, 400 μg/ml trastuzumab, or their combination using RIPA lysis buffer (Thermo Fisher Scientific, USA). A total of 50 μg of protein from each sample was resolved in 8—12% polyacrylamide gel and subsequently transferred to a PVDF membrane (Life Technologies, USA). The membranes were incubated overnight at 4 °C with primary antibodies against extracellular signal-regulated kinase (ERK1/2) (Cat# sc-514302), phosphoinositide 3-kinase (PI3K) (Cat# sc-374534), phospho-p38 (Cat# sc-166182), and β-actin (Cat# sc-69879) (all antibodies were from Santa Cruz Biotechnology, Inc., USA). Detection of bound antibodies was achieved

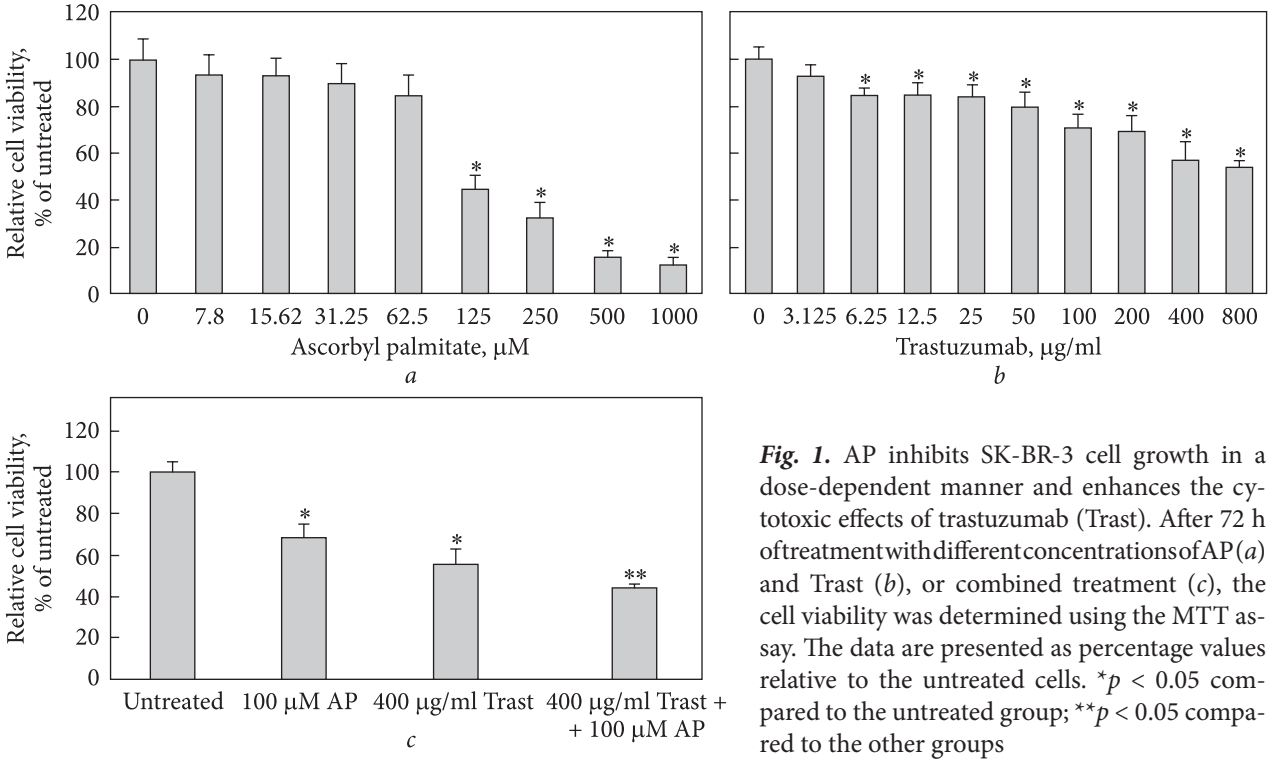

Fig. 1. AP inhibits SK-BR-3 cell growth in a dose-dependent manner and enhances the cytotoxic effects of trastuzumab (Trast). After 72 h of treatment with different concentrations of AP (*a*) and Trast (*b*), or combined treatment (*c*), the cell viability was determined using the MTT assay. The data are presented as percentage values relative to the untreated cells. $^{*}p < 0.05$ compared to the untreated group; $^{**}p < 0.05$ compared to the other groups

using the appropriate IgG and a chemiluminescence Western blot substrate kit (Thermo Fisher Scientific, USA). Quantitative analysis of protein band intensity was conducted using the Bio-Rad ChemiDoc MP System (USA) [27].

Analysis of Ki-67 expression. SK-BR-3 cells were cultured in 2-well chamber slides and subjected to the treatments described earlier (3 x 10^5 cells per well). The wells were washed with PBS and incubated with 2% paraformaldehyde in PBS for fixation. Fixated cells were incubated with a permeabilization solution, including 0.5% Triton X-100 and 1% bovine serum albumin (BSA). Then, the cells were blocked with 5% BSA and incubated overnight with the primary Ki-67 antibody (Cat# E-AB-60601, Elabscience, USA) at 4 °C. On the following day, the cells were washed with PBS, then incubated with the secondary antibody (Cat#AB150077, Elabscience, USA) for 1 h at room temperature in the dark and stained with DAPI (Abcam, UK). The samples were scored under a fluorescence microscope. The presence of any green-stained signals in the cells was considered positivity for Ki-67 [28].

Statistical analysis. Statistical analyses were carried out using SPSS software (version 20, SPSS Inc., Chicago, IL, USA). One-way ANOVA followed by Duncan's multiple range test was used to assess differences in viability, gene expression, and protein expression levels between untreated and treated groups. Each experiment was repeated at least three times, with results reported as mean ± standard deviation (SD). A *p*-value of less than 0.05 was considered statistically significant.

Results

AP inhibited the proliferation of HER2-positive BC cells. To determine the effective dose, SK-BR-3 cells were treated with 7.8 μM to 1000 μM AP for 72 h, and their effects on the cell growth were assessed. As shown in Fig. 1, *a*, high doses of AP inhibited cell growth in a dose-dependent manner. Similarly, cell viability was determined by the MTT assay 72 h after exposure to trastuzumab at the doses ranging from 3.125 to 800 μg/mL. Both agents significantly reduced cell viability in a dose-dependent manner compared to the untreated groups (Fig. 1, *a*, *b*). Subsequently, the approximate median doses of AP (100 μM) and trastuzumab (400 μg/mL) were selected and simultaneously applied to the cells to determine any potential increase in cytotoxic activity. The combined treatment significantly reduced cell viability more than the use of AP and trastuzumab individually (Fig. 1, *c*). The microscopic images of the treated cells were consistent with the MTT-test cell viability data (Fig. 2).

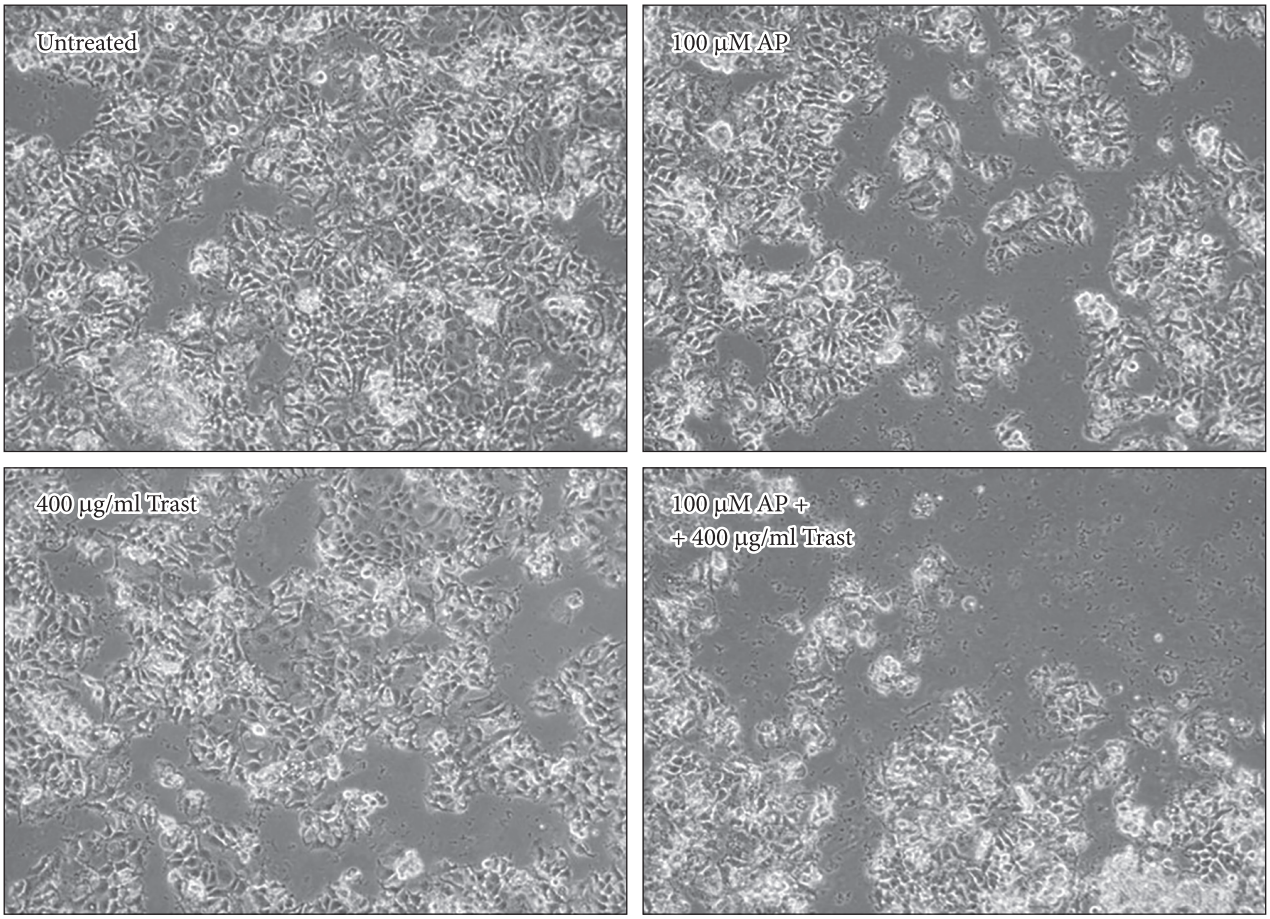

Fig. 2. Combination treatment reduces cancer cell viability. The morphology of BC SK-BR-3 cells was examined under a microscope after 72 h of treatment with 100 μM AP, 400 μg/mL trastuzumab (Trast), or their combination

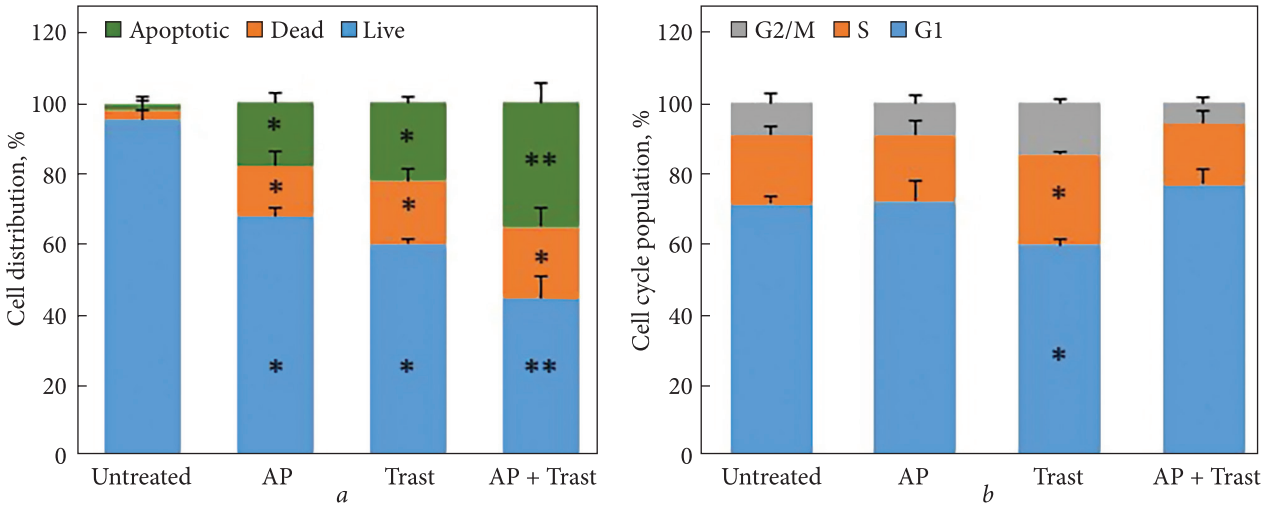

Fig. 3. Effects of AP and trastuzumab (Trast) on apoptosis (*a*) and cell cycle distribution (*b*) in SK-BR-3 cells. Cells were exposed to 100 μM AP, 400 μg/mL Trast, or a combination of both for 72 h. Apoptosis and cell cycle distribution were assessed using cytometric analysis. *$p < 0.05$ compared to the untreated group; **$p < 0.05$ compared between treatments

Combined application of AP and trastuzumab induced apoptosis. The involvement of the apoptotic cell death in the cytotoxic activity induced by treatments was analyzed using flow cytometry. According to the results, while the individual application of either agent induced apoptosis compared to the control, the combined treatment significantly enhanced this effect. The results show that AP and

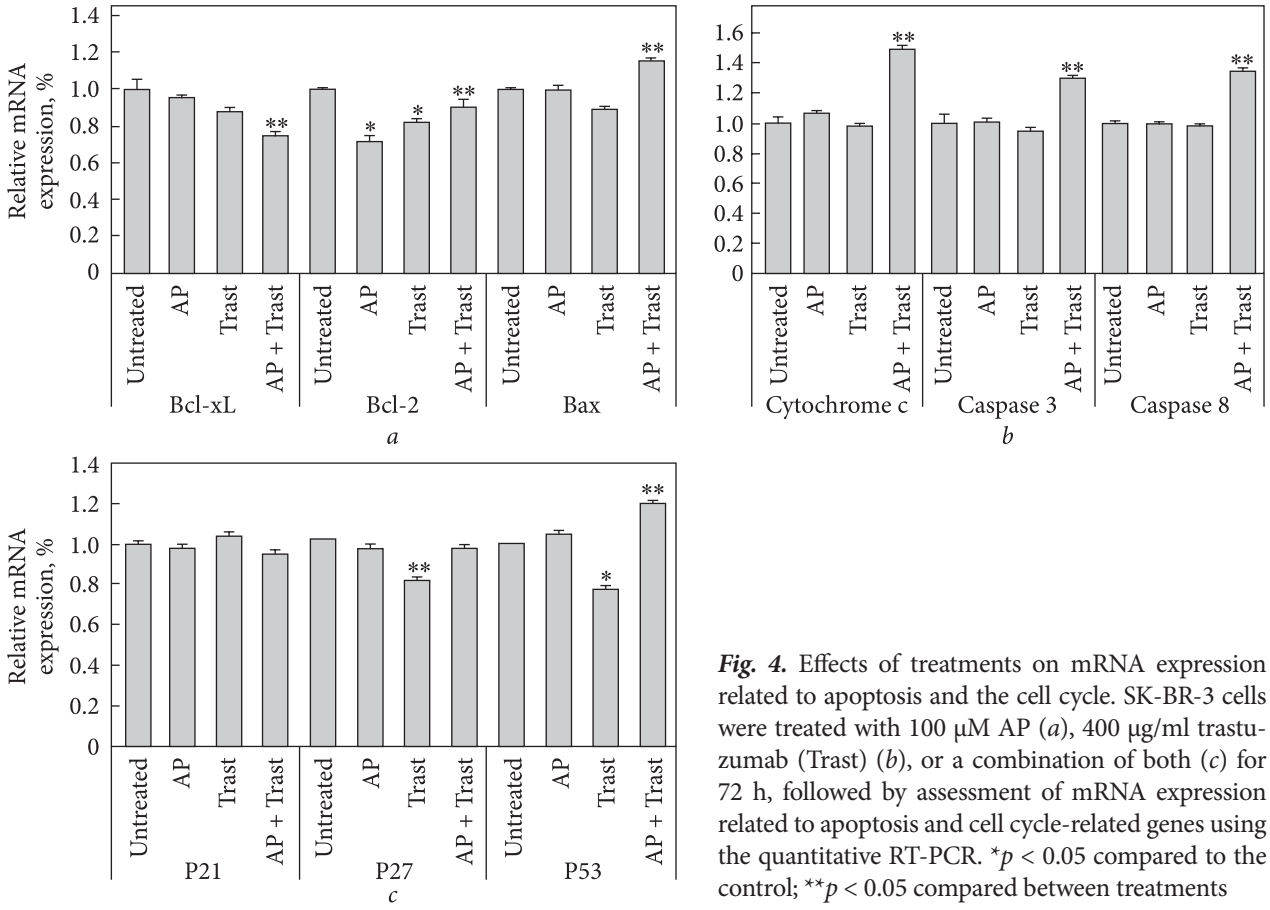

Fig. 4. Effects of treatments on mRNA expression related to apoptosis and the cell cycle. SK-BR-3 cells were treated with 100 μM AP (*a*), 400 μg/ml trastuzumab (Trast) (*b*), or a combination of both (*c*) for 72 h, followed by assessment of mRNA expression related to apoptosis and cell cycle-related genes using the quantitative RT-PCR. $^{*}p < 0.05$ compared to the control; $^{**}p < 0.05$ compared between treatments

trastuzumab induced apoptosis in 18% and 22% of cells, respectively, while their combination — in 35% of cells (Fig. 3, *a*). Regardless of the type of treatment, all applications caused approximately 14%—18% of non-apoptotic cell death compared to the untreated group (Fig. 3, *a*).

The potential effect of the treatments on the cell cycle was assessed. Trastuzumab arrested the cell cycle at the G2/M transition, while AP had no effect. In the combined treatment, the effectiveness of trastuzumab was lost (Fig. 3, *b*).

Modulation of mRNA expression. The molecular mechanisms underlying the apoptosis induced by treatments were determined through mRNA expression analysis of apoptosis-related genes using the quantitative RT-PCR. According to the results, AP and trastuzumab significantly downregulated the expression of the anti-apoptotic gene *BCL2L1*, with the combination treatment decreasing it by 25% compared to the control (Fig. 4, *a*). Additionally, *BCL2* mRNA expression was reduced by 28% with AP and 18% with trastuzumab, while the combination treatment reduced it by 10%. On the other hand, the pro-apoptotic gene *BAX* expression did not show significant changes under single treatments but showed a significant 15% increase under the combined treatment (Fig. 4, *a*).

As shown in Fig. 4, *b*, the individual treatments did not cause significant changes in the expression of the genes coding for cytochrome c, caspase 3, and caspase 8, while the combined treatment resulted in increases by 51, 30, and 35%, respectively. Similarly to the cytometry cell cycle data, the mRNA analyses of the cell cycle-related genes *CDKN1A* and *CDKN1B* also yielded comparable results. The treatments did not cause significant changes in the expression levels of these two genes, except for trastuzumab, which reduced *CDKN1B* expression by 15% (Fig. 4, *c*). Additionally, while trastuzumab decreased *TP53* expression by 21%, the combined treatment upregulated it by 20%.

Ki-67 expression. Cells in groups with three replicates were evaluated using fluorescent microscope images. In each group, at least 100 cells (ranging from a minimum of 140 to a maximum of 450) were recorded as positive or negative. Although there was no significant difference between the groups in terms of Ki-67 index ($p > 0.05$) (Fig. 5),

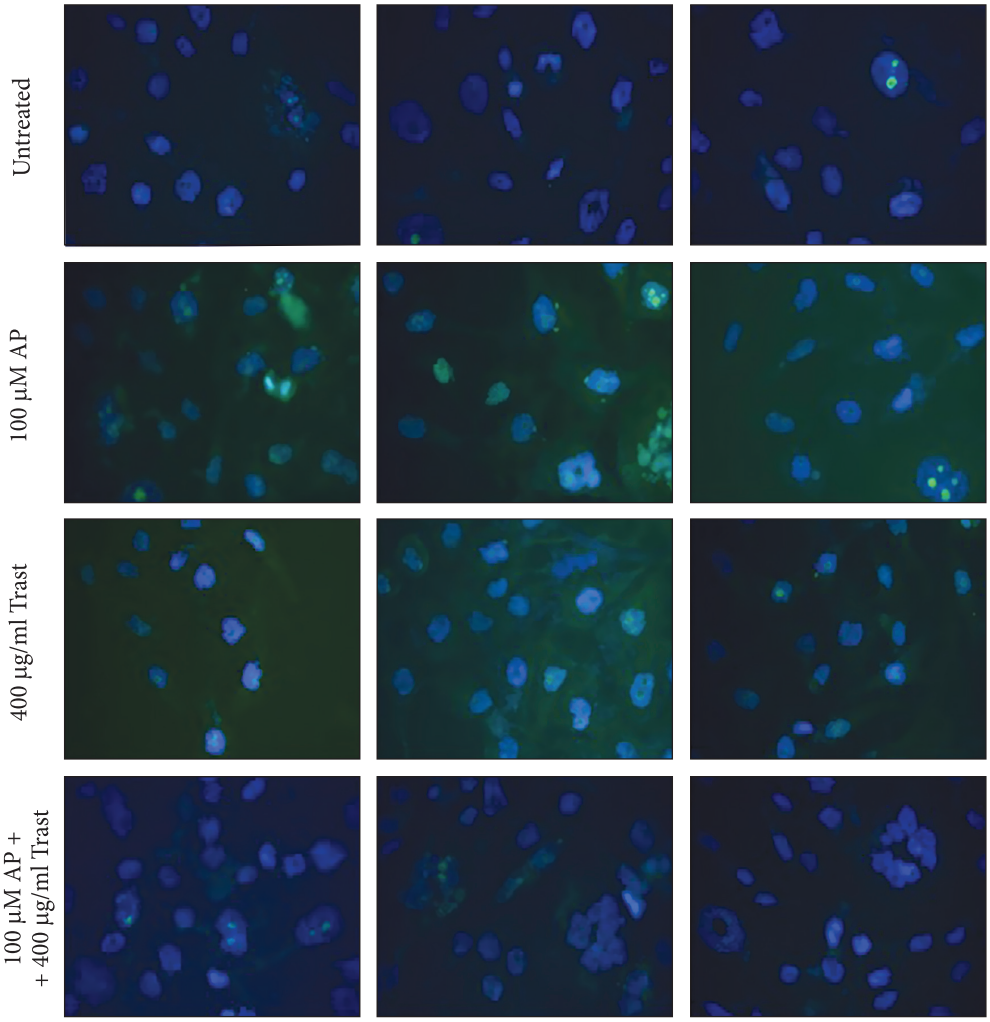

Fig. 5. Ki-67 expression analysis. SK-BR-3 cells were exposed to 100 µM AP, 400 µg/ml trastuzumab (Trast), or a combination of both for 72 h, followed by Ki-67 expression analysis using fluorescence microscopy. No significant differences were detected between the groups ($p > 0.05$)

particularly noticeable abnormal changes in nuclear morphology were observed following AP and combination treatment. These changes included cell fusion, irregularities in nuclear contours, and structures resembling micronuclei.

Regulation of protein expression. The changes in the expression of major intracellular signaling pathway proteins associated with cell survival, growth, and proliferation, namely PI3K, ERK1/2, and P38, were determined using Western blotting. While AP and trastuzumab did not cause significant changes in ERK1/2 expression individually, their combined application resulted in a 43% downregulation of this protein expression (Fig. 6, *a*). Although neither AP nor trastuzumab changed PI3K expression, their combination treatment reduced this protein by 48% (Fig. 6, *b*). Phosphorylated P38 expression was reduced by 13% and 37% in response to AP and trastuzumab treatments, respectively, compared to the untreated cells. Similarly, the combined treatment also reduced its expression by approximately 33% (Fig. 6, *c*).

Discussion

This study investigated the effects of AP and trastuzumab on HER2-positive BC cells in vitro, focusing on their impact on cell proliferation, apoptosis, Ki-67 expression, and the key intracellular signaling pathways at the molecular level. The data indicated that the combined application of these two agents resulted in a significantly greater cytotoxic effect compared to the use of each agent individually. AP effectively inhibited the proliferation of SK-BR-3 cells in a dose-dependent manner, similarly to

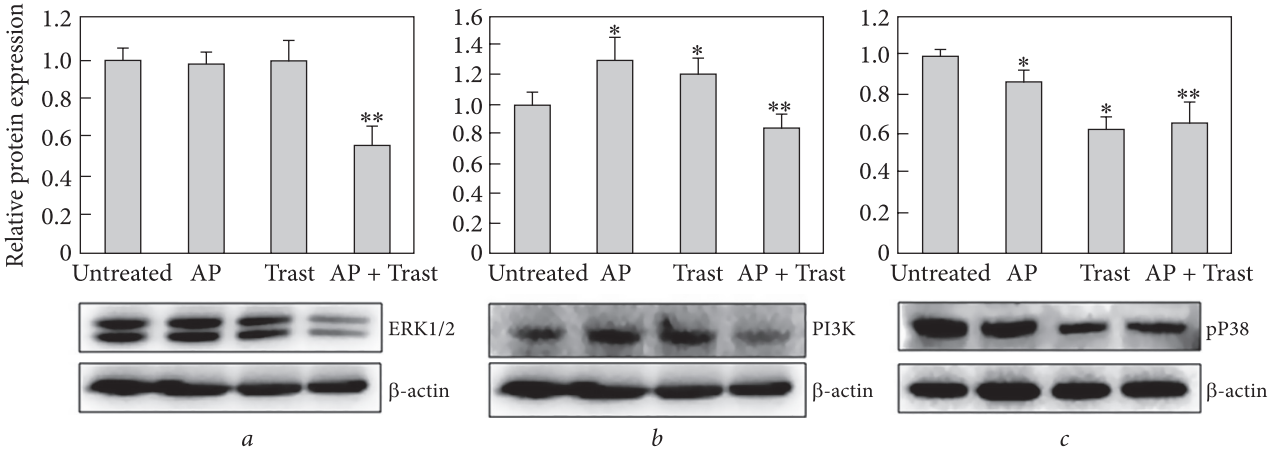

Fig. 6. Effects of treatments on the key signaling pathways. SK-BR-3 cells were exposed to 100 μM AP, 400 μg/mL trastuzumab (Trast), or a combination of both for 72 h. ERK1/2 (*a*), PI3K (*b*), and phospho-P38 (*c*) expression were then evaluated using Western blot. β-actin was used for normalization of protein expression. *$p < 0.05$ compared to the control; **$p < 0.05$ compared between the treatments

trastuzumab. The antiproliferative activity of AP, both when used alone and in combination, has been demonstrated in other studies. For example, AP-containing nanoparticle formulations kill cancer cells by increasing the production of extracellular reactive oxygen species [29]. Additionally, liposomal formulation of AP with 5-fluorouracil synergistically inhibits mouse BC cells [30]. Both agents individually reduced cell viability, consistent with previous studies showing their individual efficacies against HER2-positive BC cells [6, 15, 16, 19]. In particular, the combination of AP and trastuzumab resulted in a more pronounced decrease in cell viability compared to either agent alone. The increase in the activity is consistent with other reports suggesting that the combination of different therapeutic agents can overcome cell proliferation and enhance overall efficacy [15, 22, 31].

The first-line treatment protocol includes HER2-targeted therapy, combining chemotherapy with the monoclonal antibodies (mAbs) trastuzumab and pertuzumab. Trastuzumab is the first mAb approved by the U.S. Food and Drug Administration for treatment of HER2-positive BC [32]. The development of trastuzumab therapy has significantly improved the survival rates of women with HER2-positive BC. However, phase 3 studies indicate that the 8-year overall survival rate for patients receiving chemotherapy in combination with HER2-targeted therapy is 37% [33]. The inadequate response to HER2-directed therapy may be attributed to three characteristics of the HER2 receptor: accessibility, heterogeneity, and organization within the plasma membrane [34]. It also may be associated with disruptions in the downstream signaling pathways of the HER2 receptor. First, the changes in the PTEN-PI3K/AKT and MEK/ERK pathways [35, 36] regulate critical processes such as apoptosis, metabolism, cell proliferation, and growth. Second, the decreases in the levels of the cyclin-dependent kinase inhibitor p27 [37] regulate cell proliferation, motility, and apoptosis. Western blotting revealed that the combination of AP and trastuzumab leads to significant downregulation of key intracellular signaling proteins, including ERK1/2 and PI3K. Specifically, the combination treatment reduced ERK1/2 and PI3K expression by 43% and 48%, respectively, which is consistent with the known role of these pathways in cell survival and proliferation [38, 39]. The reduction in phosphorylated P38 expression further supported the notion that the combination therapy affects multiple signaling pathways involved in cellular stress responses [40].

The cytometry analysis revealed that both AP and trastuzumab individually induced apoptosis in HER2-positive BC cells, with the combination therapy significantly increasing the apoptotic response. Specifically, the combination therapy induced apoptosis in 35% of cells, compared to 18% and 22% with AP and trastuzumab alone, respectively. The increase in apoptosis observed with the combined treatment aligns with other research suggesting that combination therapies can amplify apoptotic pathways [15, 31]. The mRNA expression analysis revealed significant changes in apoptosis-related gene expression. The combination of AP

and trastuzumab resulted in a notable reduction in anti-apoptotic *BCL2L1* and *BCL2* and an increase in the pro-apoptotic *BAX*. These changes indicate a shift toward a pro-apoptotic environment with the combination therapy, aligning with studies that have shown the importance of balancing pro- and anti-apoptotic signals in cancer treatment [41]. Moreover, the increased expression of the genes coding for cytochrome c, caspase-3, and caspase-8 with the combination therapy suggests an enhanced activation of apoptotic pathways. This is consistent with previous findings that combined therapies can lead to higher activation of apoptotic mediators [42]. The observed changes in cell cycle-related genes, particularly the downregulation of P27 by trastuzumab and the upregulation of P53 by the combination treatment, provide further insight into the mechanistic effects of these therapies on cell cycle regulation [43].

The expression of Ki-67, a marker of cell proliferation, is a key indicator of tumor cell growth and tumor grading. According to the findings of this study, notable nuclear abnormalities, such as cell fusion and irregular nuclear contours, were observed following AP and combination treatments. These observations suggest that, despite unchanged Ki-67 levels, cellular morphology is disrupted, which may potentially impact cell function and viability [44]. There are studies suggesting that although trastuzumab is effective in targeting HER2-positive cells, Ki-67 expression levels may not show significant changes after treatment [45, 46]. This indicates that while the combination of AP and trastuzumab may improve clinical outcomes, its effect on the proliferation marker Ki-67 may be limited.

In this study, we combined AP with trastuzumab to enhance the antitumor efficacy of this monoclonal antibody. Our findings underscore the potential of such combination therapy to improve outcomes for HER2-positive BC, warranting further clinical investigation. However, a key limitation of this study is the inability to assess the efficacy of the AP and trastuzumab combination treatment in an in vivo setting.

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Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

Author contributions

SE: study design, data evaluation, RS and KT: experiments, statistical analysis.

Data availability statements

The data supporting the findings of this study are available upon request.

REFERENCES

1. IARC. Estimated age-standardized incidence rates (World) in 2020, worldwide, both sexes, all ages. Accessed 30.07.2021, 2021. https://gco.iarc.fr/today/home
2. Tsang JYS, Tse GM. Molecular classification of breast cancer. *Adv Anat Pathol*. 2020;27(1):27-35. https://doi.org/10.1097/PAP.0000000000000232
3. Godoy-Ortiz A, Sanchez-Munoz A, Chica Parrado MR, et al. Deciphering HER2 breast cancer disease: biological and clinical implications. *Front Oncol*. 2019;9:1124. https://doi.org/10.3389/fonc.2019.01124
4. Moo TA, Sanford R, Dang C, Morrow M. Overview of breast cancer therapy. *PET Clin*. 2018;13(3):339-354. https://doi.org/10.1016/j.cpet.2018.02.006
5. Cameron D, Piccart-Gebhart MJ, Gelber RD, et al. 11 years' follow-up of trastuzumab after adjuvant chemotherapy in HER2-positive early breast cancer: final analysis of the HERceptin Adjuvant (HERA) trial. *Lancet*. 2017;389(10075):1195-1205. https://doi.org/10.1016/S0140-6736(16)32616-2
6. Ghosh R, Narasanna A, Wang SE, et al. Trastuzumab has preferential activity against breast cancers driven by HER2 homodimers. *Cancer Res*. 2011;71(5):1871-1882. https://doi.org/10.1158/0008-5472.Can-10-1872
7. Wolff AC, Hammond ME, Hicks DG, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol*. 2013;31(31):3997-4013. https://doi.org/10.1200/JCO.2013.50.9984
8. Fan Y, Li X, Zhong P, et al. Radiological features for predicting the status of cD8-positive lymphocytes in HER2 positive breast cancer. *Balkan Med J*. 2024;41(3):213-221. https://doi.org/10.4274/balkanmedj.galenos.2024.2024-2-64

9. Fiszman GL, Jasnis MA. Molecular mechanisms of trastuzumab resistance in HER2 overexpressing breast cancer. *Int J Breast Cancer*. 2011;2011:352182. https://doi.org/10.4061/2011/352182
10. Lemmon MA, Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell*. 2010;141(7):1117-34. https://doi.org/10.1016/j.cell.2010.06.011
11. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol*. 2001;2(2):127-137. https://doi.org/10.1038/35052073
12. Alzahrani AS. PI3K/Akt/mTOR inhibitors in cancer: At the bench and bedside. *Semin Cancer Biol*. 2019;59:125-132. https://doi.org/10.1016/j.semcancer.2019.07.009
13. Pencik J, Pham HT, Schmoellerl J, et al. JAK-STAT signaling in cancer: From cytokines to non-coding genome. *Cytokine*. 2016;87:26-36. https://doi.org/10.1016/j.cyto.2016.06.017
14. Tebbutt N, Pedersen MW, Johns TG. Targeting the ERBB family in cancer: couples therapy. *Nature Rev Cancer*. 2013;13(9):663-673. https://doi.org/10.1038/nrc3559
15. Zhou M, Li X, Li YY, et al. Ascorbyl palmitate-incorporated paclitaxel-loaded composite nanoparticles for synergistic anti-tumoral therapy. *Drug Deliv*. 2017;24(1):1230-1242. https://doi.org/10.1080/10717544.2017.1370619
16. Mustafi S, Camarena V, Qureshi R, et al. Vitamin C sensitizes triple negative breast cancer to PI3K inhibition therapy. *Theranostics*. 2021;11(8):3552-3564. https://doi.org/10.7150/thno.53225
17. Banks WA, Kastin AJ. Peptides and the blood-brain-barrier - lipophilicity as a predictor of permeability. *Brain Res Bull*. 1985;15(3):287-292. https://doi.org/10.1016/0361-9230(85)90153-4
18. Lee SJ, Jeong JH, Lee IH, et al. Effect of high-dose vitamin c combined with anti-cancer treatment on breast cancer cells. *Anticancer Res*. 2019;39(2):751-758. https://doi.org/10.21873/anticanres.13172
19. Gu X, Zhu D, Jiang Y, et al. Adoption of ultrasound-guided polymer nanocarriers in tumor chemoradiotherapy and oxidation treatment. *Cell Mol Biol (Noisy-le-grand)*. 2023;69(2):138-143. https://doi.org/10.14715/cmb/2023.69.2.23
20. Naidu KA, Tang JL, Naidu KA, et al. Antiproliferative and apoptotic effect of ascorbyl stearate in human glioblastoma multiforme cells: modulation of insulin-like growth factor-I receptor (IGF-IR) expression. *J Neuro-Oncol*. 2001;54(1):15-22. https://doi.org/10.1023/A:1012545311054
21. Kageyama K, Onoyama Y, Kimura M, et al. Enhanced inhibition of DNA-synthesis and release of membrane phospholipids in tumor-cells treated with a combination of acylated ascorbate and hyperthermia. *Int J Hyperther*. 1991;7(1):85-91. https://doi.org/10.3109/02656739109004979
22. Kalaycioglu GD. Preparation of magnetic nanoparticle integrated nanostructured lipid carriers for controlled delivery of ascorbyl palmitate. *MethodsX*. 2020;7:101147. https://doi.org/10.1016/j.mex.2020.101147
23. Roomi MW, House D, Tsao CS. Cytotoxic effect of substitution at 2-, 6-, and 2,6-positions in ascorbic acid on malignant cell line. *Cancer Biochem Biophys*. 1998;16(4):295-300.
24. Kato S, Asada R, Kageyama K, et al. Anticancer effects of 6-o-palmitoyl-ascorbate combined with a capacitive-resistive electric transfer hyperthermic apparatus as compared with ascorbate in relation to ascorbyl radical generation. *Cytotechnology*. 2011;63(4):425-435. https://doi.org/10.1007/s10616-011-9363-8
25. Guvenc A, Ustundag K, Yoruyus A, et al. Homoeriodictyol inhibits survival and migration of androgen-resistant prostate cancer cells in vitro. *Exp Oncol*. 2025;47(1):34-43. https://doi.org/10.15407/exp-oncology.2025.01.034
26. Erdogan S, Turkekul K, Dibirdik I, et al. Midkine silencing enhances the anti-prostate cancer stem cell activity of the flavone apigenin: cooperation on signaling pathways regulated by ERK, p38, PTEN, PARP, and NF-kappaB. *Invest New Drugs*. 2020;38(2):246-263. https://doi.org/10.1007/s10637-019-00774-8
27. Erdogan S, Serttas R, Dibirdik I, Turkekul K. Multifaceted impact of adipose conditioned media: Obesity-driven promotion of prostate cancer and cancer stem cell dynamics. *Cell Biochem Funct*. 2024;42(2):e3979. https://doi.org/10.1002/cbf.3979
28. Zhang Y, Han Y, Dong J, Li F, Sun Y. Asiaticoside down-regulates HIF-1alpha to inhibit proliferation, migration, and angiogenesis in thyroid cancer cells. *Balkan Med J*. 2024;41(1):23-29. https://doi.org/10.4274/balkanmedj.galenos.2023.2023-7-123
29. Sawant RR, Vaze OS, Wang T, et al. Palmitoyl ascorbate liposomes and free ascorbic acid: comparison of anticancer therapeutic effects upon parenteral administration. *Pharm Res-Dordr*. 2012;29(2):375-383. https://doi.org/10.1007/s11095-011-0557-8
30. Katharotiya K, Shinde G, Katharotiya D, et al. Development, evaluation and biodistribution of stealth liposomes of 5-fluorouracil for effective treatment of breast cancer. *J Liposome Res*. 2022;32(2):146-158. https://doi.org/10.1080/08982104.2021.1905661
31. Li J, Guo C, Feng F, et al. Co-delivery of docetaxel and palmitoyl ascorbate by liposome for enhanced synergistic antitumor efficacy. *Sci Rep*. 2016;6:38787. https://doi.org/10.1038/srep38787
32. Giordano SH, Temin S, Chandarlapaty S, et al. Systemic therapy for patients with advanced human epidermal growth factor receptor 2-positive breast cancer: ASCO Clinical Practice Guideline Update. *J Clin Oncol*. 2018;36(26):2736-2740. https://doi.org/10.1200/JCO.2018.79.2697
33. Swain SM, Baselga J, Kim SB, et al. Pertuzumab, trastuzumab, and docetaxel in HER2-positive metastatic breast cancer. *N Engl J Med*. Feb 19 2015;372(8):724-374. https://doi.org/10.1056/NEJMoa1413513

34. Maddox AL, Brehove MS, Eliato KR, et al. Molecular assessment of HER2 to identify signatures associated with therapy response in HER2-positive breast cancer. *Cancers (Basel)*. 2022;14(11)https://doi.org/10.3390/cancers14112795
35. Berns K, Horlings HM, Hennessy BT, et al. A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer. *Cancer Cell*. 2007;12(4):395-402. https://doi.org/10.1016/j.ccr.2007.08.030
36. Nagata Y, Lan KH, Zhou X, et al. PTEN activation contributes to tumor inhibition by trastuzumab, and loss of PTEN predicts trastuzumab resistance in patients. *Cancer Cell*. 2004;6(2):117-127. https://doi.org/10.1016/j.ccr.2004.06.022
37. Nahta R, Takahashi T, Ueno NT, et al. P27 down-regulation is associated with trastuzumab resistance in breast cancer cells. *Cancer Res*. 2004;64(11):3981-3986. https://doi.org/10.1158/0008-5472.Can-03-3900
38. Erdogan S, Turkekul K, Dibirdik I, et al. Midkine downregulation increases the efficacy of quercetin on prostate cancer stem cell survival and migration through PI3K/AKT and MAPK/ERK pathway. *Biomed Pharmacother*. 2018;107:793-805. https://doi.org/10.1016/j.biopha.2018.08.061
39. Zhang H, Zhang L, He Y, et al. PI3K PROTAC overcomes the lapatinib resistance in PIK3CA-mutant HER2 positive breast cancer. *Cancer Lett*. 2024;598:217112. https://doi.org/10.1016/j.canlet.2024.217112
40. Khajah MA, Mathew PM, Luqmani YA. Inhibitors of PI3K/ERK1/2/p38 MAPK show preferential activity against endocrine-resistant breast cancer cells. *Oncol Res*. 2017;25(8):1283-1295. https://doi.org/10.3727/096504017x14883245308282
41. Campone M, Noel B, Couriaud C, et al. c-Myc dependent expression of pro-apoptotic Bim renders HER2-overexpressing breast cancer cells dependent on anti-apoptotic Mcl-1. *Mol Cancer*. 2011;10:110. https://doi.org/10.1186/1476-4598-10-110
42. Menendez JA, Vellon L, Lupu R. Antitumoral actions of the anti-obesity drug orlistat (XenicalTM) in breast cancer cells: blockade of cell cycle progression, promotion of apoptotic cell death and PEA3-mediated transcriptional repression of Her2/neu (erbB-2) oncogene. *Ann Oncol*. 2005;16(8):1253-1267. https://doi.org/10.1093/annonc/mdi239
43. Schneider J, Jeon YW, Suh YJ, Lim ST. Effects of ruxolitinib and calcitriol combination treatment on various molecular subtypes of breast cancer. *Int J Mol Sci*. 2022;23(5):2535. https://doi.org/10.3390/ijms23052535
44. Shabo I, Svanvik J, Lindstrom A, et al. Roles of cell fusion, hybridization and polyploid cell formation in cancer metastasis. *World J Clin Oncol*. 2020;11(3):121-135. https://doi.org/10.5306/wjco.v11.i3.121
45. Kute TE, Savage L, Stehle JR, Jr., et al. Breast tumor cells isolated from in vitro resistance to trastuzumab remain sensitive to trastuzumab anti-tumor effects in vivo and to ADCC killing. *Cancer Immunol Immunother*. 2009;58(11):1887-1896. https://doi.org/10.1007/s00262-009-0700-0
46. Mayer IA. Treatment of HER2-positive metastatic breast cancer following initial progression. *Clin Breast Cancer*. 2009;9(Suppl 2):S50-S57. https://doi.org/10.3816/CBC.2009.s.005

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АСКОРБІЛ ПАЛЬМІТАТ ПОСИЛЮЄ АНТИПРОЛІФЕРАТИВНИЙ ЕФЕКТ ТРАСТУЗУМАБУ ДЛЯ HER2-ПОЗИТИВНИХ КЛІТИН РАКУ ГРУДНОЇ ЗАЛОЗИ

Стан питання. На рак молочної залози (РМЗ) припадає близько 30% усіх випадків захворювань на рак у жінок, а смертність складає біля 15%. HER2-позитивний РМЗ, що є агресивним підтипом, складає 15—20% усіх випадків РМЗ. Протипухлинний ефект високих доз вітаміну С обумовлений збільшенням активності реактивних форм кисню в ракових клітинах, що не супроводжується значною токсичністю. **Мета** роботи полягала в дослідженні in vitro ефекту аскорбілпальмітату (АП), ліпофільного похідного вітаміну С, та трастузумабу, блокатора рецептора HER2, на клітини РМЗ. **Матеріали та методи.** HER2-позитивні клітини SK-BR-3 культивували в присутності АП та трастузумабу, окремо або разом. Визначали виживаність клітин за допомогою МТТ-тесту, апоптоз та розподіл за клітинним циклом за допомогою проточної цитометрії, експресію мРНК та білків за допомогою Вестерн-блот-аналізу та ЗТ-кПЛР. **Результати.** АП дозо- та часозалежно знижує виживаність клітин. Цей ефект посилюється трастузумабом. Сумісне застосування цих двох агентів посилює апоптоз в клітинах. Збільшення експресії мРНК *TP53, BAX*, а також генів, які кодують цитохром с, каспазу-3 та каспазу-8 поряд зі зниженням експресії *BCL-2* та *BCL2L1* також свідчить про індукцію апоптотичних процесів. Про антипроліферативну ефективність застосованих засобів свідчить також пригнічення експресії білків фосфо-P38, ERK1/2 та PI3K. **Висновок.** Одержані дані свідчать про потенційну ефективність комбінованого/спільного застосування АП та трастузумабу для лікування РМЗ.

Ключові слова: рак грудної залози, трастузумаб, HER2-позитивність, аскорбілпальмітат, апоптоз.