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Cytotoxic and antioxidant effects of paclitaxel and glutathione combination on breast cancer cell line

Paklitaksel ile glutatyon kombinasyonunun meme kanseri hücre hattında sitotoksik ve antioksidan etkileri

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Abstract

Aim: The aim of this study was to investigate the effects of glutathione (GSH) on chemotherapy-related toxicities in MCF-7 breast cancer cell line treated with paclitaxel (PTX) by cell viability and oxsidative stres parameters.

Materials and Methods: Cells were treated with glutathione (2.5-20 mM) and paclitaxel (0.001-100 µM) for 24, 48 and 72 hours, after which cell viability was determined by WST-1 assay. IC_{50} values were calculated from the data obtained. Following combination analyses, the combination index was calculated and the levels of glutathione, total oxidant species (TOS) and total antioxidant species (TAS) were measured in cell lysates exposed to the indicated combinations for 72 hours.

Results: In the study, it was determined that the cytotoxic effect of paclitaxel decreased as the amount of glutathion used in the combinations increased and glutathion concentrations above 10 mM showed antagonistic effect with paclitaxel.

Conclusion: In patients with breast cancer, the administration of appropriate doses of glutathione in combination with chemotherapy may prove beneficial in reducing the adverse effects associated with oxidative stress.

Keywords: Antioxidant, Glutathione, Cancer, MCF-7, Paclitaxel.

Öz

Amaç: Bu çalışmanın amacı Paklitaksel (PTX) ile tedavi edilen MCF-7 meme kanseri hücre hattında glutatyonun (GSH) kemoterapi ile ilişkili toksisiteler üzerindeki etkilerini hücre canlılığı ve oksidatif stres parametreleri ile araştırmaktır.

Gereç ve Yöntem: Hücreler 24, 48 ve 72 saat boyunca glutatyon (2,5-20 mM) ve paklitaksel (0,001-100 µM) ile muamele edilmiş, ardından hücre canlılığı WST-1 testi ile belirlenmiştir. Elde edilen verilerden IC_{50} değerleri hesaplanmıştır. Kombinasyon analizlerinin ardından kombinasyon indeksi hesaplanmış ve 72 saat boyunca belirtilen kombinasyonlara maruz bırakılan hücre lizatlarında glutatyon, toplam oksidan türler (TOS) ve toplam antioksidan türler (TAS) seviyeleri ölçülmüştür.

Bulgular: Çalışmada, kombinasyonlarda kullanılan glutatyon miktarı arttıkça paklitakselin sitotoksik etkisinin azaldığı ve 10 mM üzerindeki glutatyon konsantrasyonlarının paklitaksel ile antagonistik etki gösterdiği tespit edilmiştir.

Sonuç: Meme kanseri hastalarında, kemoterapi ile birlikte uygun dozlarda glutatyon uygulanması, oksidatif stres ile ilişkili olumsuz etkileri azaltmada faydalı olabilir.

Anahtar Kelimeler: Antioksidan, Glutatyon, Kanser, MCF-7, Paklitaksel.

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Introduction

Breast cancer is the most commonly diagnosed cancer in women worldwide and can also affect men, although less frequently. Breast cancer accounts for 31% of all new cases of cancer diagnosed in women and 15% of all deaths caused by cancer. It is the second most common cause of cancer-related deaths in women worldwide.[1,2] Despite the extensive research conducted on its treatment, the desired success in reducing its high mortality and morbidity rates has not been achieved. Common treatments for breast cancer include surgery (lumpectomy or mastectomy), radiation therapy, chemotherapy, hormone therapy, targeted therapy (such as HER2-targeted drugs), and immunotherapy.[3] Although treatment options for breast cancer depend on factors such as the type and stage of the cancer, as well as the patient's general health status and preferences, chemotherapy is currently the most effective and commonly used treatment.[1,4] Chemotherapy is used either alone or in combination with surgery and/or radiotherapy in treatment protocols.[4] Various chemotherapeutic agents, including doxorubicin (DOX), cisplatin (CP), docetaxel (DTX), and paclitaxel (PTX), have been developed and are widely used for cancer treatment.[5] These chemotherapeutic agents can induce apoptosis through both extrinsic and intrinsic pathways in the cell by inducing ROS production.[6]

The use of chemotherapeutic drugs can lead to the development of secondary (therapeutic-induced) malignancies, as well as nephro-, hepato-, neuro-, cardio-, and ototoxicity. It is important to consider and prevent these side effects, which can result in a decreased quality of life.[7,8] Although chemotherapy is the preferred treatment, its efficacy is often reduced due to the lack of drug selectivity and the development of drug resistance.[9] The ideal treatment aims to stop the growth of cancer cells, prevent invasion and metastation, and to eliminate uncontrolled cancer cells without harming healthy cells. Additionally, the goal is to prolong life and reduce the complications of treatment.[10,11] To avoid these disadvantages during treatment, many protocols have been and are being studied.[12]

The effects of antioxidants in minimising the toxicities caused by chemotherapy have been the subject of curiosity.[13] The combination of paclitaxel with curcumin reduced the side effects of treatment and increased the chemosensitivity of cancer cells to paclitaxel.[5,14]

Alterations in glutathione levels are known to be involved in the pathogenesis of many human diseases, including cancer.[12] It emphasises the importance of glutathione in cancer-related studies, especially because glutathione affects the growth and division processes of cells and plays a role in DNA repair processes.[15, 24]

Glutathione (L-γ-glutamyl-L-cysteinylglycine) is a tripeptide consisting of cysteine, glutamic acid and glycine that plays a central role in several cellular processes, including cell proliferation, death and differentiation. Due to its reducing properties, glutathione (GSH) is involved several metabolic and physiological processes, including the modulation of the immune response and detoxification of xenobiotics, in addition to protein synthesis.[16-18] GSH scavenges free radicals, which can damage cells and contribute to diseases and aging.[13] Glutathione functions as an antioxidant through various mechanisms, including direct interaction with reactive oxygen species (ROS), reactive nitrogen species (RNS), and electrophiles. Its thiol group, derived from the cysteine residue, is particularly important for these antioxidant functions.[16,19] It also regenerates other antioxidants, like vitamins C and E, which enhances the body’s ability to combat oxidative damage.[20] Some studies suggest that GSH may have potential benefits in preventing and treating cancer due to its antioxidant properties and its ability to support the immune system.[17,21-24] GSH levels can affect cell proliferation and apoptosis, both of which are dysregulated in cancer. Moderate levels of GSH are essential for cell survival and proliferation. However, excessively high levels may promote cancer cell growth by inhibiting apoptosis and supporting tumor progression.[25,26] Chemotherapy resistance is a significant challenge in cancer treatment, and GSH has been implicated in this process.

Cancer cells can increase GSH synthesis to counteract the cytotoxic effects of chemotherapy drugs, resulting in treatment resistance.[21,27,28] However, several studies have suggested that GSH plays a crucial role in chemotherapy by protecting healthy cells, enhancing treatment effectiveness, and reducing side effects.[29] Some studies suggest that antioxidants may work together with anti-cancer drugs, allowing for greater and longer uptake of anti-neoplastic agents, thereby increasing the effectiveness of treatment. According to a meta-analysis, using antioxidants in conjunction with chemotherapy enhances therapeutic potential and survival rates in cancer patients.[30,31]

Cancer patients use antioxidant supplements such as glutathione, an antioxidant mixture, melatonin, N-acetylcysteine, especially vitamin A and E, Co-Q10, selenium, ellagic acid and L-carnitine acid to alleviate the side effects of chemotherapies. However, glutathione is important both as an endogenous antioxidant and because of its role in chemotherapy resistance. For these reasons, it was aimed to investigate the determination of cell viability, oxidative stress balance and intracellular glutathione level in the simultaneous use of glutathione with chemotherapy.

Materials and Methods

Culturing the cell

The MCF-7 (human breast cancer) cell line used in the study was obtained from the Republic of Turkey Ministry of Agriculture and Forestry Şap Institute. MCF-7 cell lines were incubated in 25 cm^2 flasks under a constant 5% CO_2 flow at 37ºC. Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich) F12 was supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich, Burlington, MA, USA), 4 mM L-glutamine (Gibco), 0.02 M non-essential amino acid (Sigma, M7145), 1 mM sodium pyruvate (Sigma, P5280), 16 mg/dl gentamicin.

Preparation of materials and application of WST-1 analysis

In order to optimize cell numbers for WST-1 analysis, cells are seeded in 1:2, 1:4, 1:8 and 1:16 dilutions from $2x10^4$ cells. After 24 h, viability analysis is carried out by WST-1 assay (Roche-11644807001) and results are calculated in GraphPad Prism 8. The optimal number of cells for WST-1 analysis was determined to be $5x10^3$ cells per well.

In order to carry out cytotoxic analyses of PTX, 200 µl of the cell suspension was added to each well of a 96-well plate containing $5x10^3$ cells. The plate was then incubated in 5% CO_2 for 24 hours. Subsequently, different concentrations of PTX (ranging from 0.001 to 100 µM) in serially diluted medium were added to the cells. After 24, 48, and 72 hours of incubation, medium was removed and 100 µl of medium containing 10% WST-1 was added. Cells were incubated at 37ºC 5% CO_2 for 3 hours. At the end of the incubation period, the microplate reader (Thermo scientific multiscan go microdrop) was measured at 450 nm and the results were recorded.

Different concentrations of GSH (2.5, 5, 10, 15 and 20 mM; Sigma) were prepared from a 100 mM GSH stock solution in cell medium to study the effect of GSH on cell proliferation. The cells were treated with GSH and its viability was assessed using WST-1 after 24, 48, and 72 hours of incubation.

Determination of cytotoxicity levels with Trypan Blue

$1x10^6$ cells were seeded in 6-well plates and incubated for 24 h. followed by treatment with PTX ranging from 0.001 to 100 µM and different concentrations of GSH (2.5, 5, 10, 15 and 20 mM) and incubated for 24, 48 and 72 hours. At the end of the incubation period, cells were stained with trypan blue and counted. Cells were also treated with the determined combination doses for 72 hours and counted.

The trypan blue staining assay allows direct identification and counting of live (unstained) and dead (blue) cells in a given population. Prior to the trypan blue staining procedure, adherent cells were first trypsinised and suspended in PBS. After obtaining a homogeneous cell suspension, an appropriate amount (5-10 µl) of cell culture was mixed with an equal amount of trypan blue (0.4%). The cells were then counted in appropriate quantities in a haemocytometer. Cells that took

up the dye were considered dead and cells that did not were considered alive. The total percentage of dead cells in the suspension was thus determined.[32,33]

Determination of combination indexes

After obtaining cytotoxicity results for PTX and GSH at varying concentrations and time points in the MCF-7 cell line, we calculated the 72-hour cytotoxicity results for combinations using IC_{50} doses. These results were analysed using the CompuSyn programme 1.0 (ComboSyn Inc., Paramus, NJ, USA) to calculate combination indices (CI) for standard or different targeted drug combinations. The programme's CI analysis is based on the median-effect principle.[34]

WST-1 was analysed by applying the determined combinations individually and in combination by accepting only cells and medium containing cells and medium, without any agent as a positive control.

Determination of GSH levels

The cells were incubated for 72 hours with the combinations determined according to the results. After incubation, the cells were suspended in trypsin-EDTA (Sigma T4049) and washed with PBS (Invitrogen, 003002).

The lysate obtained from the cells, which were lysed by the freeze-thaw method, was deproteinised on ice using 5% sulfosalicylic acid. The supernatants were analysed for GSH after being freeze-thawed at -196ºC and 37ºC three times.[35,36]

Total oxidant species and total antioxidant species determinations

Total antioxidant species level (TAS) and total oxidant species level (TOS) analyses were performed in cell medium with REL Assay Diagnostic colorimetric kits. TAS is a method developed by Erel to measure the total antioxidant capacity of the body against powerful free radicals. Fe^{2+}-o-dianisidine complex forms OH radical by Fenton-type reaction with H_2O_2. This powerful reactive oxygen species (ROS) reacts with the colourless odianisidine molecule at low pH to form yellow-brown dianisidyl radicals. Dianisidyl radicals participate in further oxidation reactions and increase colour formation. However, antioxidants in the samples suppress these oxidation reactions and stop the colour formation. The samples are calibrated to Trolox, a vitamin E analogue.[37]

TOS is a colorimetric method developed by Erel. Oxidants in the sample oxidise the ferrous ion-o-dianisidine complex to a ferric ion. The presence of glycerol in the medium accelerates this reaction, increasing it approximately threefold. In an acidic medium, ferric ions form a coloured complex with xylenol orange.[38]

Statistical analysis

In the analysis of cytotoxicity and the examination of combination experiments, the studies were conducted with eight repetitions. Two analytical replicates were conducted for each of the GSH, TAS and TOS analyses, with a total of three biological replicates.

Data were collected and analysed using SPSS for Windows® Version 22 software. The conformity of the variables to normal distribution was analysed by Shapiro-Wilk test. Descriptive analyses were given using mean and standard deviation for normally distributed and non-normally distributed variables. Since the data obtained as a result of WST analysis did not conform to normal distribution was used by transforming the data (logarithm was taken in combination analyses). Whether there was a statistically significant difference between PTX, GSH and their combinations treated cell groups, and control groups in terms of inhibition of cell viability was determined by 2-way ANOVA, Tukey's test.

Results

WST-1 analysis and calculation of IC_{50}

In order to determine the effect of GSH on cell proliferation, GSH was prepared in concentrations of 2.5, 5, 10, 15 and 20 mM from a 100 mM stock solution in cell medium. Viability tests were performed with WST-1 after 24, 48 and 72 hours incubation. Upon evaluation of the results, it was determined that there was a maximum of 12% inhibition on cell viability in the first 24 hours, with 50% inhibition observed in the 48th hour following

the application of 15 mM GSH. However, when the results of 20 mM GSH application were evaluated in comparison to the control group, no inhibition on cell viability was observed. IC_{50} values could not be calculated with the viability results obtained in the first 48 hours. Upon evaluation of the results obtained at the 72-hour time point, it was determined that the viability rates in cells treated with 10 mM, 15 mM, and 20 mM GSH were 58.88%, 49.22%, and 52.3%, respectively. The 72-hour IC_{50} dose of GSH was found to be 7.5 mM (Figure 1). A series of dilutions of PTX (0.001-100 µM) were prepared in a medium and added to the cells. After 24, 48 and 72 hours of incubation, a cell proliferation assay was performed with WST-1. After 24 hours of incubation, a statistically significant cytotoxic effect was observed in cells treated with 10 µM and 100 µM PTX. After 48 hours of treatment, cell viability was significantly inhibited at all concentrations, with the exception of 0.001 and 0.01 µM. At 72 hours, no cytotoxic effect was observed, except the 0.001 µM PTX treatment. The IC_{50} dose of PTX was found to be 6 µM at 72 hours (Figure 2).

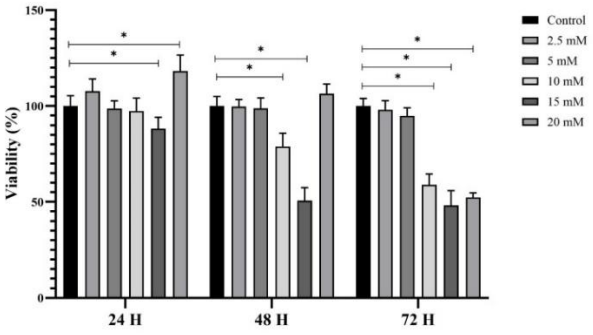

Figure 1. Results of GSH cytotoxicity in MCF-7 at 24, 48 and 72 hours (n=8, **p*<0.001).

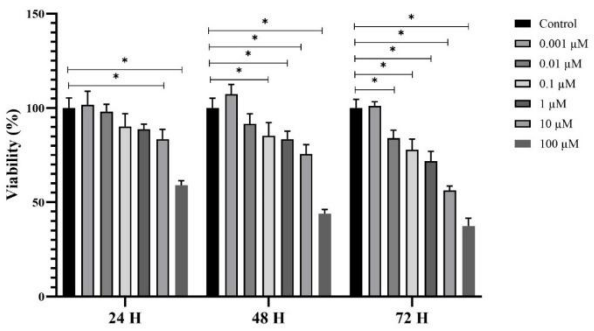

Figure 2. Results of PTX cytotoxicity in MCF-7 at 24, 48 and 72 hours (n=8, **p*<0.001).

Determination of cell viability by trypan blue staining

After 24, 48 and 72 hours of incubation, cells treated with GSH and PTX were stained with trypan blue and counted in a hemocytometer. When the results were analysed, all doses except 20 mM GSH in the first 24 hours showed an antiproliferative effect. At these doses, MCF-7 cells showed a maximum viability of 80%. In the first 48 hours, all other concentrations except 20 mM showed antiproliferative effect with a maximum cell viability of 50%. Similar results were observed at 72 h with a partial increase in antiproliferative effect (Table 1).

PTX treatment showed antiproliferative effect on cells depending on dose and duration (Table 2).

PTX and GSH combinations

Following the acquisition of cytotoxicity data for PTX and GSH at varying concentrations and time-dependent cytotoxicity data for the MCF-7 cell line, 72-hour cytotoxicity data for the combinations formed by considering IC_{50} doses were calculated (Figure 3, Table 3). 72 hours was preferred in combination applications because no significant cytotoxic effect was observed in the first 48 hours of GSH administration.

Table 1. Percentage expression of cell viability by trypan blue staining of GSH-treated MCF-7 cells after 24, 48 and 72 hours of incubation.

GSH concentration (mM)	24 H Cell viability (%) (n=8)	48 H Cell viability (%) (n=8)	72 H Cell viability (%) (n=8)
Control	100±1.52^{a}	100±2.79^{a}	100±3.15^{a}
2.5	110.84±2.45^{b}	98.74±2.35^{ab}	98.02±3.16^{a}
5	102.5±2.14^{a}	96.12±3.01^{b}	92.33±1.01^{b}
10	94.27±2.78^{c}	73.45±4.01^{c}	56.48±4.16^{c}
15	83.65±4.56^{d}	53.24±4.31^{d}	49.14±3.78^{d}
20	120.95±4.11^{e}	109.09±3.46^{e}	57.9±2.65^{c}

$^{a, b}$: Differences between means shown with different letters in the same column are statistically significant.

Table 2. Percentage expression of cell viability of PTX-treated MCF-7 cells by trypan blue staining after 24, 48 and 72 hours of incubation

PTX concentration (µM)	24 H Cell viability (%) (n=8)	48 H Cell viability (%) (n=8)	72 H Cell viability (%) (n=8)
Control	100±1.45[a]	100±3.45[a]	100±3.74[ab]
0.001	103.93±1.23[b]	109.05±2.03[b]	102.63±4.35[a]
0.01	96.38±2.47[c]	95.39±3.26[c]	86.67±4.03[b]
0.1	91.45±3.57[d]	89.14±2.97[d]	79.14±3.65[c]
1	87.17±1.69[e]	85.03±3.56[e]	69.46±3.25[d]
10	85.69±2.13[e]	71.79±4.03[f]	53.03±4.29[e]
100	53.31±3.45[f]	40.39±2.78[g]	35.13±2.38[f]

a, b: Differences between means shown with different letters in the same column are statistically significant

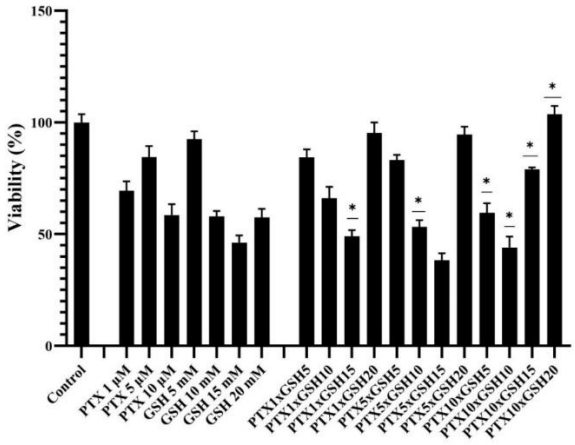

Figure 3. The results of the cytotoxic effect of PTX and GSH combinations in the MCF-7 cell line at 72 hours (PTX: Paclitaxel-µM, GSH: Glutathione-mM) (n=8, *p<0.001).

Table 3. Percentage expression of cell viability by trypan blue staining of MCF-7 cells treated with GSH and PTX combinations after 72 hours of incubation.

GSH and PTX combinations	Cell viability (%) (n=8)
Control	100±3.56
1 µM PTX	72.89±6.25
5 µM PTX	82.50±1.24
10 µM PTX	55.12±5.23
5 mM GSH	91.25±3.14
10 mM GSH	54.36±2.65
15 mM GSH	47.36±4.37
20 mM GSH	56.32±2.69
1 µM PTX + 15 mM GSH	47.39±4.25
5 µM PTX + 10 mM GSH	51.78±5.02
10 µM PTX + 5 mM GSH	57.85±2.48
10 µM PTX + 10 mM GSH	45.63±2.03
10 µM PTX + 15 mM GSH	76.78±2.74
10 µM PTX + 20 mM GSH	101.34±4.26

The data were entered into the CompuSyn programme, which automatically analyses the data of common or different targeted drug combinations, and CI was calculated. The CI analysis employed in this programme is based on the median-effect principle.[34] The CI values obtained were interpreted in accordance with the guidelines set out in Table 4. (CI<0.9=Synergism; 0.9-1.1=positive; CI>1.1= Antagonism).

Table 4. Combinations applied to MCF-7 cell culture line.

Concentrations	CI Value	Commentary*
10 µM PTX + 5 mM GSH	0.387	Strong synergism
1 µM PTX + 15 mM GSH	0.482	Synergism
10 µM PTX + 10 mM GSH	0.459	Synergism
10 µM PTX + 15 mM GSH	0.561	Synergism
5 µM PTX + 10 mM GSH	1.646	Antagonizm
10 µM PTX + 20 mM GSH	1.876	Antagonizm

* CI=1 indicates additive effect, CI<1 indicates synergistic effect and CI>1 indicates antagonism (Chou, 2010)

The combinations exhibiting a synergistic effect demonstrated a significant cytotoxic effect compared to the control (p<0.001). However, the proliferation of cells was observed in the combination with 20 mM GSH.

The effect of the determined combinations on cell viability was determined by both WST-1 and trypan blue staining. When the results obtained with the two methods were evaluated, no statistically significant difference was found between them (p>0.05).

Determination of GSH, total oxidant and total antioxidant levels

The analysis of the data revealed no significant difference in the levels of GSH in cell lysates treated with GSH and PTX (Figure 4A) (p>0.05). TAS was found to be lower in the cell line in which PTX was applied at 5 µM compared to the control (p<0.05). In contrast, GSH was found to be higher in other combinations, with the exception of the PTX10 x GSH5 combination, in which GSH was applied alone and in combination with PTX (p<0.05). The results demonstrated that GSH

levels above 5 mM had an effect on PTX toxicity (Figure 4B). PTX addition did not alter the TOS of MCF-7 cells, but it increased in cells treated with 15 and 20 mM GSH and in combinations containing GSH (p<0.05). A significant decrease was observed in the MCF-7 cell line treated with GSH10 and PTX10 x GSH10 compared to the control (p<0.05) (Figure 4C).

In summary, it was found that TAS levels increased significantly in all combination treatment groups except 5 mM GSH and 10 µM PTX combination treatment compared to the control (p<0.05). In addition, TOS levels increased significantly in all combination groups except 10 mM GSH and 10 µM PTX combination treatment compared to the control (p<0.05).

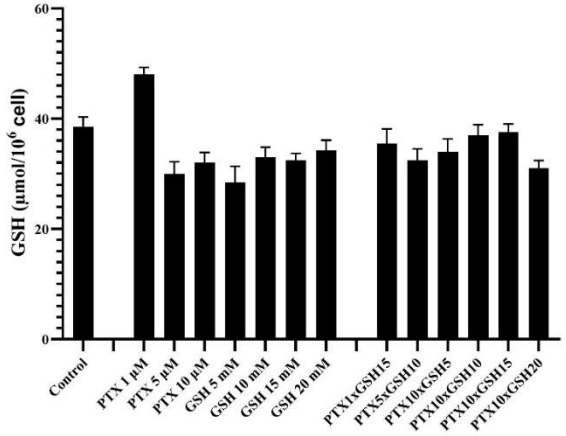

Figure 4A. GSH levels in MCF-7 cell line treated with GSH and PTX combinations for 72 hours (n=3, median ±SE).

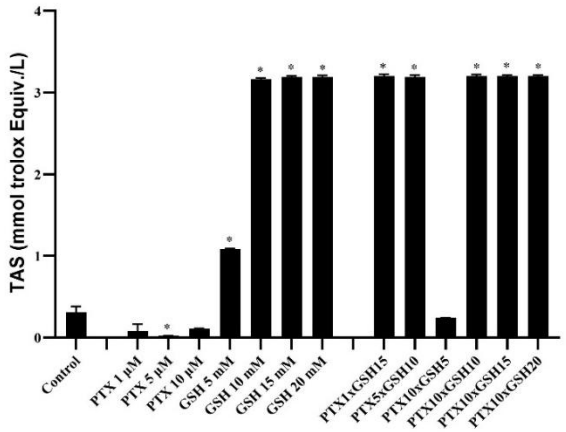

Figure 4B. TAS Level in MCF-7 cell line treated with GSH and PTX combinations for 72 hours (n=3, median ±SE). * p<0.05 different from control.

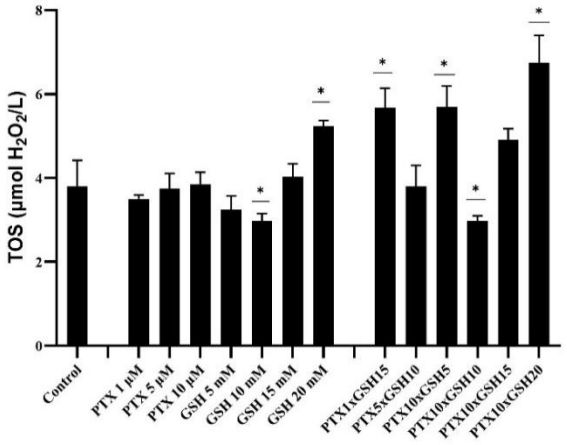

Figure 4C. TOS levels in MCF-7 cell line treated with GSH and PTX combinations for 72 hours (n=3, median±SE). * p<0.05 different from control.

Discussion

Despite the numerous new research studies and discoveries in the mechanisms of cancer and drug design, the incidence of cancer is expected to increase in the coming years. The search for natural, inexpensive treatments to prevent, treat and stop the progression of cancer has gained importance in recent years.

The objective of chemotherapeutic agents, which are currently the most effective in cancer treatment, is to destroy rapidly proliferating and growing cancer cells during the proliferative period. However, chemotherapy also affects normal cells while destroying cancer cells. Furthermore, these drugs cause an increase in ROS levels and disruption of the antioxidant balance of the cell. For this reason, the occurrence of side effects is attempted to be reduced and eliminated with the help of antioxidant substances in conjunction with chemotherapy. However, the literature still does not provide clear answers to questions such as whether the concomitant intake of antioxidants with chemotherapeutic drugs decreases the efficacy of the drug or develops drug resistance. Therefore, it is important to determine the synergistic additive and antigonistic effects of these combinations and to elucidate their mechanisms by cell culture studies. In our study, we investigated the combination of glutathione, which is important in the mechanism of chemotherapeutic drug resistance and whose intracellular concentration is controlled by many enzymes

as the same endogenous antioxidant, with paclitaxel, the most commonly used drug in breast cancer treatment.

Antioxidants, which reduce the damaging effects of free radicals, play an important role in the supportive treatment of cancer. GSH, which plays a dominant role in the antioxidant system, primarily as an intracellular radical scavenger and detoxifying molecule, is increasingly being investigated due to its potential role in the prevention and treatment of cancer.[31] This is due to the fact that the GSH system acts as a homeostatic redox buffer and is one of the primary cellular defences against free radicals.[39] GSH is crucial in the removal and detoxification of carcinogens and is thought to have a possible link with GSH deficiency in the development of cancer cells.[25,40,41] GSH deficiency or a decrease in the GSH/glutathione disulfide (GSSG) ratio leads to increased susceptibility to oxidative stress, which plays a role in cancer progression, whereas increased GSH levels increase antioxidant capacity and resistance to oxidative stress, as seen in many cancer cells.[27] The dual role of ROS in these processes further complicates the impact of GSH on oxidative stress and cancer initiation and progression.[25] The GSH system acts as a homeostatic redox buffer and is one of the first cellular defences against free radicals. Cellular redox potential is largely determined by GSH levels, which account for 90% of cellular non-protein thiols.[39] Furthermore, research has shown that increasing GSH levels in cancer cells may have anti-cancer effects by inhibiting tumour growth and promoting apoptosis or programmed cell death.[42] GSH has also been shown to increase the effectiveness of some chemotherapeutic drugs by protecting healthy cells from their toxic effects. Overall, there is growing evidence that GSH plays an important role in cancer and may have potential therapeutic effects.

In our study, PTX showed a dose- and time-dependent cytotoxic effect in MCF-7 cells. The viability assay results obtained are in parallel with similar studies.[43-45] In one study, it was observed that 1.6 mM GSH did not cause any change in the number of cells in A549 lung cells.[46] As a parallel result of this study, it was observed that GSH, which we applied at a lower concentration range (2.5-10 mM), had no significant effect on cell proliferation, but showed a cytotoxic effect similar to that of PTX as the applied dose and time increased. GSH applied at a concentration of 20 mM for 24 and 48 hours had a mitogenic effect on the cells. Alexandre et al.[46] observed that the accumulation of H_2O_2 and the cytotoxic activity of PTX against A549 cancer cells decreased with the addition of 1.6 mM N-acetylcysteine (NAC) and 1.6 mM GSH to the medium. Similarly, according to the results of the Compusyn programme in our study, GSH was found to reduce the cytotoxic effect of PTX as the amount of GSH increased in the combinations studied, and GSH above 10 mM showed an antagonistic effect with PTX.

Studies have shown that using antioxidants reduces the formation of some cancers caused by free radicals. However, as ROS have other important physiological functions, such as second messengers, it has been suggested that inhibition of apoptosis by antioxidants may prevent the destruction of unwanted (precancerous and cancerous) cells and may induce cancer in individuals with carcinogenic DNA damage.[47] Resveratrol treatment as an antioxidant caused GSH depletion in MCF-7 cells and GSH levels were found to be lower than controls.[48] A positive correlation was found between cellular levels of GSH and the growth of tumour cells in pancreatic and prostate cancer.[49,50] Studies have shown that the drug and radiation resistance of many tumours is associated with higher levels of GSH in cancer cells compared to normal tissue.[39,51] Despite an increase in ROS in MCF-7 cells treated with rose bengal, 100 mM GSH inhibited ROS generation but had no effect on toxicity.[52]

In contrast to the viability results, no significant difference was found between the intracellular GSH concentrations; GSH added to the cell media did not affect the intracellular GSH concentration. One of the limitations of our study was that we did not measure the activity of the enzyme g-glutamyl transpeptidase (GGT). While GSH synthesis occurs inside the cell, GSH degradation takes place with GGT expressed on the cell surface.

The glutamate, cysteine and glycine produced by GSH degradation are used for intracellular GSH synthesis. GGT enzyme activity is an important parameter that can provide information on the level of utilisation of GSH precursors in the extracellular fluid.[53,54].

PTX has been reported to induce the production of endogenous ROS.[45,55-57] In certain studies, it has been demonstrated that the application of PTX to cells results in an increase in ROS levels within the cell. The addition of antioxidants, such as NAC and GSH, to the medium has been shown to prevent the accumulation of ROS caused by PTX.[45,46]

A positive correlation was observed between total antioxidant capacity and PTX IC_{50} value in 16 cell lines, including MCF-7 cells. The higher the PTX IC_{50} value of tumour cells, the higher the total antioxidant capacity was found.[44] It was observed that taxol-induced apoptosis in chronic myeloid leukemia K562 cells treated with taxol, a taxane derivative, was associated with ROS production and GSH consumption. Adding NAC antioxidant to the medium was found to suppress taxol-induced apoptosis and ROS production.[58] Furthermore, in addition to the literature, it was found that TAS were considerably higher in cells and combinations that had been treated with GSH. There was also a significant increase in TOS levels. The high TAS levels indicate that GSH has a positive effect on the increase in antioxidant capacity.

Shen et al[59] investigated the effects of GSH on the chemotherapeutic efficacy of DOX in cancer cell models including MCF-7, HepG2 and Caco-2 cells. They reported that GSH administration dose-dependently decreased the anticancer efficacy of DOX both in vivo and in vitro. Therefore, they reported that the combination of GSH and DOX during chemotherapy can generally be considered contraindicated.

In the literature, many natural products have been combined with paclitaxel to reduce the side effects. The mechanisms of action of the combinations especially on cancer cell lines have been tried to be elucidated. Combinations of apigenin,[60] baicalein,[61] daidzein,[62] fisetin,[63] genistin[62] luteolin[64] have been examined and data have been obtained to reduce the side effects of apoptosis induction. However, since there are few publications with glutathione, which is an endogenous antioxidant, the results of our study make an important contribution to the literature.

Taxane family toxicity is associated with ROS production in cancer cells, leading to apoptosis activation. Cancer cells, in turn, induce an antioxidant response as a taxane resistance enhancing effect.[65] Therefore, an excess of antioxidants in the environment may actually minimize ROS production and cancer cell apoptosis. Our results show that the combination of exogenous GSH and PTX as treatment is dose dependent. It has been shown that appropriate GSH concentration increases treatment efficacy and sensitizes the cell to chemotherapeutic drugs. However, we can state that high concentrations decrease the treatment response.

Conclusion

The interactions between chemotherapeutic agents and antioxidants are complex and factors such as dose, localisation and metabolism of the drug influence the production of free radicals. Some antioxidants also have the potential to act as oxidative molecules, depending on their use and/or relative concentration. It is clear that monitoring all the enzymes and molecules involved in GSH metabolism will be more revealing in order to clearly see the antioxidant effect in cancer cells, and in this context studies are needed to investigate the multifaceted effect of GSH on cancer cells. The effectiveness of oral GSH supplements in supporting cancer treatment has not been fully established. Although GSH is an essential antioxidant, the body's ability to absorb it orally is limited and its effect on cancer treatment outcomes remains unclear.

Our results emphasise that antioxidants and chemotherapeutic agents should be used at appropriate doses and within a certain period of time. It should be kept in mind that the use of glutathione as an antioxidant in patients receiving paclitaxel chemotherapy is dose

dependent and high dose applications should be avoided.

Limitations

Although there is promising evidence suggesting a role for GSH in the prevention and treatment of cancer, most research to date has been limited to experiments using tumour cell lines or animal models. Further research is therefore needed, particularly through clinical trials in patients, to determine the efficacy and safety of using GSH as a preventive or therapeutic agent in cancer.

Ethics Committee Approval

Ethical approval was not required as this study did not involve animal or human experimentation.

Author Contributions

GSEA: Design and Conception, Resources, Materials, Analysis and/or Interpretation, Data Collection and/or Processing, Literature Review, Writing. IB: Data Collection and/or Processing, Literature Review, Analysis. AY: Literature Review, Analysis. AB: Idea/Concept, Supervision/Consultancy, Funding, Literature Review, Critical Review. PAU: Supervision/Counselling, Resources, Literature Review, Critical Review.

Conflict of interest

The authors declare that there is no conflict of interest.

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