Original Article

DOI: 10.14235/bas.galenos.2021.6306
Bezmialem Science 2022;10(4):461-9

Effects of Sulfur Containing Glycine Imine Derivatives Compounds on Multidrug Resistance Proteins (MRPs) and Apoptosis Mechanism in MCF-7 and DLD-1 Cell Lines

Kükürt İçeren Gilisin İmin Türevi Bileşiklerin MCF-7 ve DLD-1 Hücre Hatlarında Çoklu İlaç Direnci Proteinleri (MRP'ler) ve Apoptoz Mekanizması Üzerine Etkileri

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ABSTRACT

Objective: Glutathione (GSH) is a tripeptide consisting of glycine, glutamic acid and cysteine. If the sulfur containing amino acids like methionine and cysteine increase in the cells, the level of GSH increases. GSH is one of the most important and powerful antioxidants in the body and it reduces oxidative stress. Reduction of GSH affects apoptosis activity and multidrug resistance proteins (MRPs). In the present study, we investigated the effects of sulfur-containing glycine imine derivatives on MRPs and apoptosis mechanism in the MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines.

Methods: In MCF-7 and DLD-1 cell lines; mRNA levels of MRPs (ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2), apoptosis mechanism proteins (BAX, BACL-2, P53, PARP, CASP3), heat shock proteins (HSPs) and endoplasmic reticulum chaperone proteins (GRPs) were determined by qRT-PCR method.

Results: Compounds decreased gene expression of multiple drug resistance (MDR) genes and increased gene expression of pro-apoptosis mechanism genes (BAX, P53, CASP3). HSPs and BCL-2 and PARP gene expressions decreased. There was no significant decrease in gene expression of GRPs. The compounds were shown

ÖZ

Amaç: Glutatyon (GSH), glisin, glutamik asit ve sisteinden oluşan bir tripeptiddir. Metiyonin ve sistein gibi kükürt içeren amino asitler hücrelerde artarsa GSH seviyesi yükselmektedir. GSH vücuttaki en önemli ve güçlü antioksidanlardan biridir ve oksidatif stresi azaltmaktadır. GSH'nin azaltılması, apoptoz aktivitesini ve çoklu ilaç direnci proteinlerini (MRP'ler) etkilemektedir. Bu çalışmada, kükürt içeren glisin imin türevlerinin, MCF-7 (meme kanseri) ve DLD-1 (kolon kanseri) hücre hatlarında çoklu ilaç direnci proteinlerine (MRP'ler) ve apoptoz mekanizmasına etkisini araştırmayı amaçladık.

Yöntemler: MCF-7 ve DLD-1 hücre hatlarında; çoklu ilaç direnci proteinleri (ABCB1, ABCC3, ABCC10, ABCC11 ve ABCG2), apoptoz mekanizması proteinleri (BAX, BACL-2, P53, PARP, CASP3), ısı şoku proteinleri (HSP'ler) ve endoplazmik retikulum (ER) GRP'ler mRNA seviyeleri qRT-PCR yöntemi ile belirlendi.

Bulgular: Bileşikler çoklu ilaç direncinde (MDR) gen ekspresyonunu azalttı ve pro-apoptoz mekanizması genlerinde (BAX, P53, CASP3) ekspresyonu artırdı. HSP'ler, BCL-2 ve PARP gen ekspresyonları azaldı. GRP gen ekspresyonunda önemli bir azalma olmadı. Bileşiklerin, ABCC3 dışındaki çoklu ilaç direnci genleri (ABCB1,

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Cite this article as: Mesci S, Yazgan B, Gül M, Yıldırım T. Effects of Sulfur Containing Glycineimine Derivatives Compounds on Multidrug Resistance Proteins (MRPs) and Apoptosis Mechanism in MCF-7 and DLD-1 Cell Lines. Bezmialem Science 2022;10(4):461-9

Received: 12.04.2021
Accepted: 02.07.2021

to have remarkable effects on MDR genes (ABCB1, ABCC10, ABCC11 and ABCG2) other than ABCC3. Compounds were found to have significant effects on apoptosis mechanism genes and HSPs.

Conclusion: Our results indicate that the sulfur-containing glycine imine derivatives can be a potent option as a cancer drug, especially in breast and colon cancers.

Keywords: MCF-7, DLD-1, sulfur, glycineimine, MRPs, apoptosis

ABCC10, ABCC11 ve ABCG2) üzerinde önemli etkilere sahip olduğu gösterilmiştir. Ayrıca bileşiklerin apoptoz mekanizması genleri ve HSP'ler üzerinde önemli etkilere sahip olduğu tespit edilmiştir.

Sonuç: Sonuçlarımız, sülfür içeren glisin imin türevlerinin, özellikle meme ve kolon kanserinde anti-kanser ajanı olabilecek önemli ve güçlü bir seçenek olabileceğini göstermektedir.

Anahtar Sözcükler: MCF-7, DLD-1, kükürt, glisinimin, MRP'ler, apoptoz

Introduction

Glycine (NH_2CH_2COOH), which is unique because it has no isomeric forms and is a structural unit for many proteins, has been the subject of numerous studies (1). Sulfur-containing amino acids play major roles in synthesis, structure, and function of proteins (2). Sulfur is a critical element and it is found in the structure of cysteine and methionine, and it is necessary for the biosynthesis of sulfolipids, antioxidants, cofactors, secondary metabolites and amino acids (3,4). Glutathione [g-glutamyl-L-cysteinylglycine; (GSH)] containing glycine and cysteine amino acids is the strongest antioxidant in the body. It is found in almost all tissues (at concentrations of 1-10 mM) (4-6). GSH is a necessary endogenous tripeptide for the prevention of neoplasms, liver disorders, corneal disorders, eczema, heavy metal poisoning, and to prevent the negative effects of radiation therapy (7,8). Supplementing of component amino acids (cysteine, glycine and glutamate) increases GSH synthesis in tissue. Studies have reported that high GSH concentration is effective against cellular damage, tissue degeneration and disease progression (8,9). GSH is a protein that fights oxidative stress and regulates cell proliferation, apoptosis, signal transduction, gene expression, insulin resistance, immune function and fibrogenesis (4,10,11). It has been reported that negative changes in GSH homeostasis cause obesity, cancer, AIDS, diabetes mellitus and heart disease (8,12) and GSH regulates cell death. GSH level affects the expression and activity of caspases and signal molecules that are important in cell death (12). GSH levels decrease during apoptosis. Many studies suggest that GSH output through the cell during apoptosis is associated with multiple drug resistance proteins (MRP) (4,13-15).

Several members of the MRP family, ABCC1 (MRP1), ABCC2 (MRP2), ABCC4 (MRP4), ABCC5 (MRP5) and ABCC7 (CFTR) have been shown to mediate GSH transportation (14). MRPs can transport structurally and mechanically different drugs, including natural anticancer drugs, nucleoside analogues, antimetabolites and tyrosine kinase inhibitors (16). MRPs are included in the ABCC subfamily of the ABC transporter family (17,18). Expression of ABCC proteins is seen in most human tissues (18). P-glycoprotein (P-gp; ABCB1), the first discovered member of the ABC (ATP-binding cassette) transporter family, is a membrane protein that works as an efflux pump (19-21). Proteins specific to breast tissue; ABCB1 (MDR1-P-gp), ABCC11 (MRP8), ABCC12 (MRP9), ABCG2 (BCRP) and colon tissue-specific proteins; ABCB1 (MDR1-P-gp), ABCC3 (MRP3), ABCC10 (MRP7), ABCC11 (MRP8), ABCC13 (Pseudogene), ABCG2 (BCRP) belong to the ABC family (18,22).

The p53 protein is a tumor suppressor gene consisting of 393 amino acids weighing 53 kDa. The p53 gene makes a choice between life and death (apoptosis) (23-25). Generally, p53 is upregulated or mutated in MRPs of cancer cells (26,27). Intracellular BCL-2/BAX ratio is extremely important in determining whether the cell will go to apoptosis. If BAX is too much, the cell will go to apoptosis, or if BCL-2 is too much, apoptosis will be inhibited (28-30). Activation of effector caspases such as Caspase-3 causes downstream of PARP and similar substrates. Thus, it initiates apoptotic cell death (31). PARP down regulation suppresses cell proliferation and causes apoptosis via the p53 signal pathway (32). HSPs play a role as molecular chaperones that ensure the correct folding of the newly synthesized proteins or stress-dependent misfolded proteins and also prevent aggregation of the proteins (33-35). Expression of HSPs plays a role in regulating apoptosis, immune response to tumors, and multiple drug resistance. Increased HSPs levels make cells more resistant to apoptosis (36,37). Glucose regulated proteins (GRPs) are molecular chaperones in cancer tissues that are upregulated by cellular response to various stressful conditions such as glucose deprivation, oxidative stress and hypoxia (38-40). At the present time, the elimination of drug resistance (41,42) and increasing the ability to direct resistant cells to apoptosis (43,44) are the main targets for research and development of anti-cancer agents.

In this study, it was aimed to investigate the effects of sulfur containing glycine imine derivatives compounds on MRPs and apoptosis mechanism in MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines.

In our previous study, three different compounds were synthesized from glycine imine containing sulfur. Antioxidant and cytotoxic activity studies of the compounds were carried out using five different methods. The compounds were shown to exhibit antioxidant and cytotoxic activities in MCF-7 and DLD-1 cells (45). As a continuation of the study, the effects of sulfur-containing glycine imine derivatives on MRPs and apoptosis mechanisms in MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines. In our study, we showed that sulfur glycine imine-derived compounds had the ability to direct drug-resistant cells to apoptosis and exhibited significant activity in MCF-7 and DLD-1 cells.

Methods

In MCF-7 and DLD-1 cell lines; mRNA levels of MRPs (ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2), apoptosis mechanism proteins (BAX, BACL-2, P53, PARP, CASP3), heat shock proteins (HSPs) and endoplasmic reticulum (ER) chaperone proteins (GRPs) were determined by qRT-PCR method.

Cell Culture

In this study, *in vitro* studies were used for MCF-7 (ATCC® HTB22™) (breast adenocarcinoma) DLD-1 (colon cancer) (ATCC® CCL221™) human cell lines. MCF-7 and DLD-1 cells were cultured using 25 g/100 mL sodium bicarbonate, 10% fetal bovine serum (FBS) RPMI-1640 (Roswell Park Memorial Institute) mediums containing 1 % penicillin/streptomycin in 25 cm^2 or 75 cm^2 flask, 5% CO_2 and it was produced by incubation for 24 hoursat 37 °C. Stock solutions were made in dimethyl sulfoxide (DMSO) (Sigma, Steinheim, Germany) and were made with culture medium. Controls were cultured in RPMI 1,640 medium with a final concentration of 0.1% of DMSO but without compounds (46).

qRT-PCR Assay

Total RNA Isolation

The MCF-7 (breast cancer) and DLD-1 (colon cancer) cells were incubated at 37 ºC for 24 hours, they were added to the 6 plates using a number of $2x10^6$ cells. Cells with compound dose determined as 50 µM according to MTT results were washed with 1X PBS at the end of 24 hours and 1X trypsin was removed with EDTA. In order to evaluate expression at the gene level, mRNA extraction was studied in the control and dose groups in accordance with the method recommended in the kit (Thermo Fisher; RNA isolation kit [K0731]). The concentration and purity of the isolated RNA were carried out with the aid of the nanodrop device. In the process of measuring the RNA samples with nanodrop, the RNA samples were diluted at appropriate concentrations (ng/µL) and they were measured at 260 and 280 nm (µg/mL) = [optical density 260x dilution rate x 40 (µg/mL)] (A260/A280≅2.0). These RNA samples were included in the study.

cDNA Synthesis

The complementary chains of isolated total RNAs were converted into cDNA by synthesizing them with "Thermo Fisher; cDNA synthesis kit (K1622)" using oligo d (T) primer and reverse transcriptase enzyme (RT). In the 1st stage of cDNA synthesis; it was kept at 65 °C for 5 minutes. In the 2nd stage; it was kept for 1 hour at 42 °C and 5 minutes at 70 °C to inhibit the enzyme after incubation. Synthesized cDNAs were kept at -20 °C for qPCR.

qReal Time PCR (qRT-PCR)

The sequences of genes were determined by NCBI database. In line with the accessory numbers listed in the Table 1, the primers required for real-time PCR were designed with the help of the NCBI primary blast program (https://www.ncbi.nlm.nih.gov/tools/primer-blast). (Thermo Fisher; K0221); Syber Green Master Mix (2x) 5 µL Forward primer (10 mM) 1 µL, Reverse primer (10 mM) 1 µL, cDNA 2 µL and Nuclease free water 1 µL, using Piko Real 96 (Thermo Scientific) device and by applying the amplification program, mRNA expression levels were examined. All samples were studied in duplicate. After real time PCR, the melt curve analysis was performed and the specificity of the primers was tested.

Results

Chemistry

We performed this study to understand MRPs and apoptosis mechanism of ethyl 2-((bis(propylthio)methylene)amino)acetate (1), ethyl 2((bis(ethylthio)methylene)amino)acetate (2), and ethyl 2-((bis(methylthio)methylene)amino) acetate (3) on the two different cancer cell lines (Figure 1).

Glycineimine Derivatives Compounds Regulate mRNA Expression of MRPs

In MCF-7 cells; *ABCB1* gene expression was reduced by 0.6-fold, ABCC10 gene expression by 0.6-fold, *ABCC11* gene expression by 0.5-fold, and *ABCG2* gene expression by 0.3-fold with compound **I** compared with the untreated control. *ABCB1* gene expression was reduced by 0.6-fold, *ABCC10* gene expression by 0.6-fold and *ABCC11* gene expression by 0.4-fold with compound **II** compared with the untreated control. *ABCB1* gene expression was reduced by 0.7-fold, *ABCC10* gene expression by 0.4-fold, *ABCC11* gene expression by 0.5-fold, and *ABCG2* gene expression by 0.2-fold with compound **III** compared with the untreated control.

In DLD-1 cells; *ABCB1* gene expression was reduced by 0.4-fold, ABCC10 gene expression by 0.6-fold and *ABCG2* gene expression by 0.8-fold with compound **I** compared with the untreated control. ABCB1 gene expression was reduced by 0.2-fold and ABCG2 gene expression by 0.7-fold with compound **II** compared with the untreated control. ABCB1 gene expression was reduced by 0.7-fold, *ABCC10* gene expression by 0.4-fold and *ABCG2* gene expression by 0.2-fold with compound **III** compared with the untreated control.

Compound **I** reduced the expression of all other *MDR* genes except ABCC3 compared to negative control in the MCF-7 cells. It was found that expression was decreased in other genes except ABCC3 and ABCC11 in DLD-1 cells . Compound **II** reduced expression in only *ABCB1* and *ABCC11* genes by half in MCF-7 cells. In DLD-1 cells, expression was decreased only in *ABCB1* and *ABCG2* genes. Compound **III** reduced expression in ABCB1, ABCC11 and *ABCG2* genes by half in MCF-7 cells. Expression in *ABCB1*, *ABCC10* and *ABCG2* genes in the DLD-1 cells decreased significantly (Figure 2).

Glycineimine Derivatives Compounds Regulate mRNA Expression of HSPs and Apoptotic Genes

Compared to untreated control; gene expression of BAX was increased by 2-fold and p53 by 4.3-fold, and gene expression

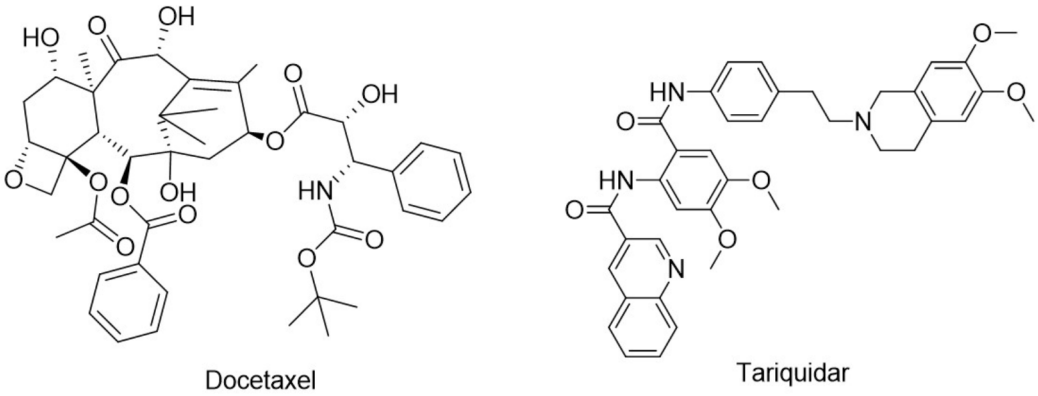
Table 1. Primer sequences, references, and product lengths

Genes	Primer sequences (5'-3')	References	Product length	Melting temperature
β-Actin F	TGACGTGGACATCCGCAAAG	NM_001101.5	205	51
β-Actin R	CTGGAAGGTGGACAGCGAGG			
ABCB1 F	GTTCAGGTGGCTCTGGATAAG	NM_001348946.1	93	55
ABCB1 R	AGCGATGACGTCAGCATTAC			
ABCC3 F	TACTCCAAGACAGAGACAGAGG	NM_003786.4	111	53
ABCC3 R	CCGGTAGCGCACAGAATAAT			
ABCC10 F	TCACCCTGTCTCCACTGTAT	NM_001198934.1	133	49
ABCC10 R	AACTGGCACCTCTGGTTTAG			
ABCC11 F	GTGGTGCTGATCGTCTTCTT	XM_017023801.2	106	53
ABCC11 R	CCATGGTTCCATTGCTCTCT			
ABCG2 F	TCGTACTGGGACTGGTTATAGG	XM_017008852.2	101	53
ABCG2 R	GTTGGTCGTCAGGAAGAAGAG			
BAX F	TCATGGGCTGGACATTGGAC	NM_001291428.1	114	59
BAX R	GAGACAGGGACATCAGTCGC			
BCL2 F	ATCTGGGCCACAAGTGAAGTC	NM_000633.2	209	59
BCL2 R	TTCGACGTTTTGCCTGAAGAC			
PARP F	GAATGCCAGCGTTACAAGCC	NM_001618.4	212	59
PARP R	TCTCCCTGAGACGTATGGCA			
P53 F	GTTTTCCCCTCCCATGTGCTC	NM_001126112.2	170	53
P53 R	CAGTCTGGCCAATCCAGGGAAG			
CASP3 F	GCGGTTGTAGAAGAGTTTCGT	NM_001354777.1	146	59
CASP3 R	TTATTAACGAAAACCAGAGCGCC			
HSP27 F	GAGGAGCATAAAAGCGCAGC	NM_001540.5	347	60
HSP27 R	CTAACCACTGCGACCACTCC			
HSP40 F	GAGGGGTTGTGAATGCAGGAG	NM_001300914.1	246	59
HSP40 R	CTCAGCAAACATGGCATGAGG			
HSP60 F	GACGACCTGTCTCGCCG	NM_002156.5	258	60
HSP60 R	GGACTTCCCCAACTCTGCTC			
HSP70 F	AAGGAGACAGCCGAAAGTGT	L12723.2	212	57
HSP70 R	CTTGGTTTCTCTTCTAAGCGAGG			
HSP90 F	GCGCTAGCAGGAGATGGTTA	NM_005348.3	103	59
HSP90 R	TTTCTGTGCCTACGTGTGCT			
GRP78 F	GAACGTCTGATTGGCGATGC	NM005347.5	143	59
GRP78 R	ACCACCTTGAACGGCAAGAA			
GRP94 F	GCCAGTTTGGTGTCGGTTTC	NM_003299.3	168	59
GRP94 R	GGGTAATTGTCGTTCCCCGT			

of BCL-2 was decreased by 0.2-fold, PARP by 0.5-fold, HSP60 by 0.5-fold, HSP70 by 0.8-fold, and HSP90 by 0.7-fold with compound **I** in MCF-7 cells. Gene expression of p53 was increased by 3.2-fold and gene expression of HSP27 was decreased by 0.5-fold, HSP40 by 0.9-fold, and HSP90 by 0.7-fold withcompound **I** in DLD-1 cells.

Compared to untreated control; gene expression of BAX was increased by 3-fold, Caspase-3 by 3-fold, p53 by 14.4-fold, and gene expression of BCL-2 was decreased by 0.8-fold, PARP by 0.2-fold, HSP60 by 0.3-fold, and HSP70 by 0.7-fold with compound **II** in MCF-7 cells. Gene expression of p53 was increased by 3.8-fold and gene expression of HSP40 was decreased by 0.8-fold, and HSP90 by 0.4-fold with compound **II** in DLD-1 cells.

Compared to untreated control; gene expression of Caspase-3 was increased by 2.7-fold, and p53 by 9.5-fold, and gene expression of BCL-2 was decreased by 0.9-fold, PARP by 0.6-fold, HSP40 by 0.5-fold, HSP60 by 0.4-fold, HSP70 by 0.8-fold, and HSP90



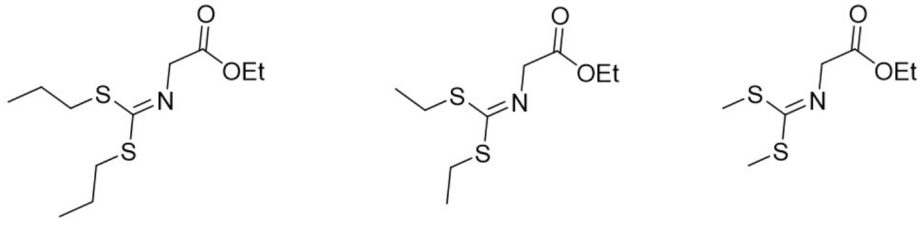


Figure 1. Docetaxel, Tariquidar chemotherapeutic drug (include aminoaside ester units) and alkyl substituted sulfur-containing glycine imine (**I**, **II**, and **III**)

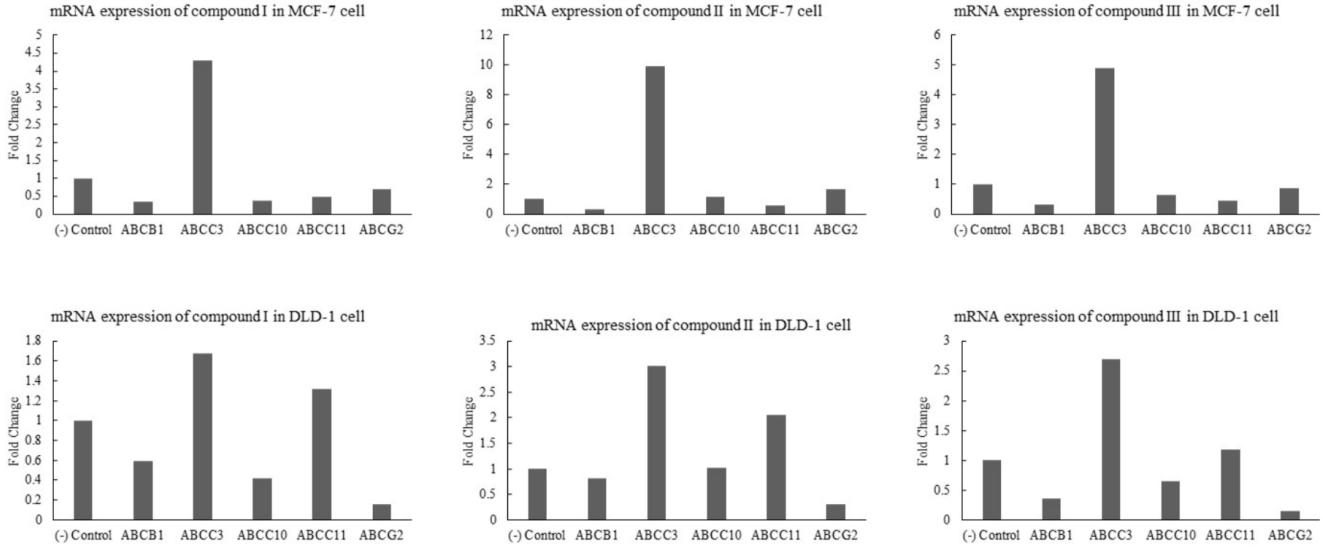


Figure 2. Glycine imine derivatives compounds regulate multidrug resistance in MCF-7 and DLD-1 cell lines. mRNA expressions of ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2 in cells are measured by qRT-PCR, normalized to β-actin

by 0.5-fold with compound **III** in MCF-7 cells. Gene expression of p53 was increased by 1.8-fold, and gene expression of PARP was decreased by 0.3-fold, HSP70 by 0.5-fold, and HSP90 by 0.3-fold with compound **III** in DLD-1 cells.

Compound **I** increased the expression of p53, BAX, CASP3 and GRPs in the MCF-7 cells. BCL-2 reduced the expression of HSPs other than PARP and HSP27. Expression of p53 was increased in DLD-1 cells and the expression of HSPs was decreased except HSP60. Compound **II** increased the expression of p53, BAX and CASP3 in the MCF-7 cells. It only reduced HSP60 and HSP70 expressions. Expression of p53 was increased in DLD-1 cells. HSP40, HSP70 and HSP90 expressions were decreased. Compound **III** increased the expression of p53 and CASP3 in the MCF-7 cells. BCL-2 reduced the expression of HSPs other than PARP and HSP27. Expression of p53 was increased in DLD-1 cells. PARP, HSP27 and HSP90 expressions were decreased. Compounds (**I**, **II** and **III**) did not have a significant effect on *GPRs* genes' (GRP78 and GRP94) expression (Figure 3).

Discussion

In our previous study, it was demonstrated that sulfur-containing glycine imine derived compounds had antioxidant and cytotoxic activities in MCF-7 and DLD-1 cell lines (45). It was stated that the compounds gave parallel results with other studies in both antioxidant and cytotoxic activities. As a continuation of the study, the effects of sulfur-containing glycine imine derivatives on MRPs and apoptosis mechanisms were determined in MCF-7 (breast cancer) and DLD-1 (colon cancer) cell lines. It was observed that biological activity studies were limited in the literature reviews of glycine imine derived compounds containing sulfur. Biological activity studies with these compounds were carried out for the first time by our group.

GSH, (2S) -2-amino-5 - [[(2R) -1- (carboxymethylamino) -1-oxo-3-sulfanylpropan-2-yl] amino] -5-oxopentanoic acid, and tripeptide are found in all tissues. GSH consists of L-cysteine, L-glutamic acid and glycine, forming L-gamma-glutamyl-Lcysteinylglycine amino acids (47). GSH in cancer cells is important in the regulation of mutagenic mechanisms, DNA synthesis, growth, and multidrug and radiation resistance (48). GSH showed inhibition of apoptosis in HeLa (49), in HSC-2 cancer cell lines (50), and in Calu-6 lung cancer cells (51). GSH levels decrease rapidly during apoptosis, and this is known as a biochemical feature of oxidant-induced programmed cell death (52). GSH inhibits the induction of apoptosis in human lung cancer cells via down-regulation of survivin (inhibitor of the apoptosis protein family). In drug-resistant cells, GSH protects healthy cells from oxidative stress, but prevents induction of apoptosis in tumor cells (53). In addition, GSH describes a new pathway of apoptosis dependent on PKC-delta activation and independent of p53, Bcl-2 and Bax levels (54). Fico et al. (13), 2008 reported that 2-deoxy-D-ribose (dRib) induced apoptosis by activating an oxidative stress by consuming GSH. In our study, the effects of sulfur containing glycine imine derived compounds on apoptosis mechanism proteins (BAX, BACL-2, P53, PARP, CASP3), HSPs and ER GRPs in MCF-7 and DLD-1 cell lines. The compounds decreased expression in anti-apoptotic (*HSPs*, *BCL-2* and *PARP*) genes, while increased expression in pro-apoptotic (BAX, P53, PARP) genes. Compound **I** increased the expression of p53, BAX, CASP3 and GRPs in MCF-7 cells. BCL-2 decreased the expression of HSPs other than PARP and HSP27. Expression of p53 was increased in DLD-1 cell and expression of HSPs was decreased. Compound **II** increased p53, BAX and CASP3 expressions in MCF-7 cells, as well as decreased HSP60 and HSP70 expressions. In DLD-1 cells, p53 expression was increased, moreover HSP40, HSP70 and HSP90 expressions were decreased. Compound **III** increased p53 and CASP3 expressions in MCF-7 cells. BCL-2 effectively reduced the expression of PARP and HSPs. In DLD-1 cells, p53 expression was increased, furthermore PARP, HSP27 and HSP90 expressions were decreased. Decreasing anti-apoptotic gene expressions and increasing pro-apoptotic gene expressions drive cancer cells to apoptosis. The data of the study determined that the compounds decreased HSPs, BCL-2 and PARP gene expressions and increased BAX, P53, CASP3, PARP gene expressions, leading MCF-7, and DLD-1 cancer cell lines to apoptosis.

In studies, Hammond et al. (14), 2007 showed evidence that GSH was released through MRP1 during both internally and externally induced apoptosis. In our study, the effects of sulfur containing glycine imine derived compounds multidrug resistance proteins (ABCB1, ABCC3, ABCC10, ABCC11 and ABCG2) in MCF-7 and DLD-1 cell lines were investigated. The compounds decreased expression in multidrug resistance (ABCB1, ABCC10, ABCC11 and ABCG2) genes. Compound **I** effectively reduced the expression of MDR genes in the MCF-7

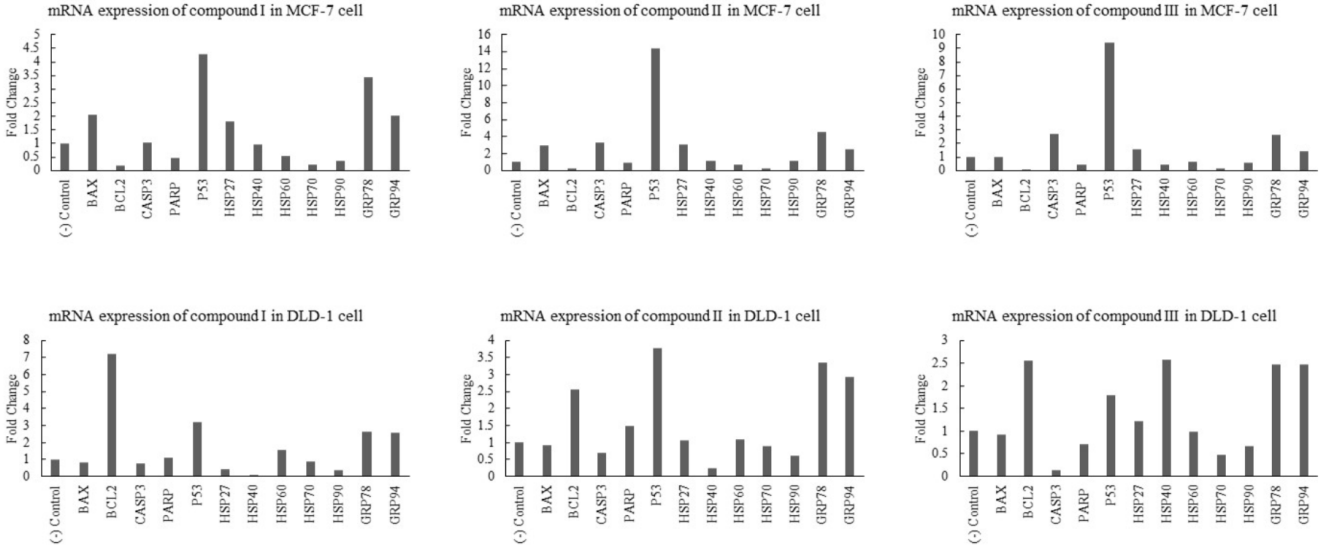


Figure 3. Glycine imine derivatives compounds regulate apoptosis mechanism, HSPs and GRPs in MCF-7 and DLD-1 cell lines. mRNA expressions of apoptosis mechanism, HSPs and GRPs in cells are measured by qRT-PCR, normalized to β-actin

cell compared to the negative control. Compound **I** reduced the expression of *ABCB1*, *ABCCC10* and *ABCG2* genes in DLD-1 cells compared to the negative control. Compound **II** reduced expression of *ABCB1* and *ABCC11* genes by half in MCF-7 cells . Expression of ABCB1 and ABCG2 genes was decreased in DLD-1 cells . Compound **III** halved the expression in the ABCB1, ABCC11 and ABCG2 genes in the MCF-7 cells. Expression in the ABCB1, ABCC10 and ABCG2 genes significantly decreased in DLD-1 cells. The expression of MRPs in the cell membrane prevents cancer cells from drifting into apoptosis. With the compounds used in the study, the expression of proteins that caused drug resistance was suppressed and it was proven that cancer cells could drift into apoptosis.

Conclusion

In brief, in our study, compounds increased gene expression in MDR and apoptosis mechanism genes (BAX, P53, PARP). HSPs and BCL-2 and PARP gene expressions decreased. There was no significant decrease in gene expression of GRPs. The compounds (**I**-**II**-**III**) were shown to have remarkable effects on ABCB1, ABCC10, ABCC11 and ABCG2 genes. Compounds **I**, **II**, and **III** were found to have no effect on the ABCC3 gene in MCF-7 and DLD-1 cells. No significant effect of the compounds (**I**, **II** and **III**) on GPRs genes (GRP78 and GRP94) was detected. Compounds (**I**-**II**-**III**) were found to have significant effects on apoptosis mechanism genes and heat shock proteins (HSPs). Compared to DLD-1 cells, the effects of the compounds on MCF-7 cells were more marvelous.

According to the results of the used compounds on MDR in cells, they had the ability to direct cancer cells to apoptosis. The compounds used in the study were useful for anticancer studies. Derivatives of the compounds were thought to contribute to further studies. Our results showed that sulfur glycine imine derivatives had a potent ability to direct resistant cells to apoptosis, although MCF-7 cells exhibited remarkable activity.

Acknowledgments: The authors thank the Amasya University Central Research Laboratory (AUMAULAB) for their kind understanding of using their facilities.

Ethics

Ethics Committee Approval: The article is a cell culture study. It does not require an ethics committee.

Informed Consent: No patients were used in the study. Informed consent is not required.

Peer-review: Externally peer reviewed.

Authorship Contributions

Surgical and Medical Practices: S.M., B.Y., M.G., T.Y., Concept: M.G., T.Y., Design: M.G., T.Y., Data Collection or Processing: S.M., B.Y., M.G., Analysis or Interpretation: S.M., B.Y., M.G., T.Y., Literature Search: S.M., B.Y., M.G., Writing: S.M., B.Y., M.G., T.Y.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This work was supported by Amasya University (FMB-BAP 18-0333).

References

1. Akinde DO. Amino acid efficiency with dietary glycine supplementation: Part 1. World's Poultry Science Journal 2014;70:461-74.
2. Brosnan JT. Brosnan ME. The Sulfur-Containing Amino Acids: An Overview. J Nutr 2006;136(6 Suppl):1636S-40.
3. Abadie C, Tcherkez G. Plant sulphur metabolism is stimulated by photorespiration. Commun Biol 2019;2:379.
4. Lu SC. Glutathione synthesis. Biochim Biophys Acta 2013;1830:3143-53.
5. Metges CC, Yu YM, Cai W, Lu XM, Wong S, Regan MM, et al. Oxoproline kinetics and oxoproline urinary excretion during glycine- or sulfur amino acid-free diets in humans. Am J Physiol Endocrinol Metab 2000;278:E868-76.
6. Forman HJ, Zhang H, Rinna A. Glutathione: overview of its protective roles, measurement, and biosynthesis. Mol Aspects Med 2009;30:1-12.
7. Alanazi AM, Mostafa GA, Al-Badr AA. Glutathione. Profiles Drug Subst Excip Relat Methodol 2015;40:43-158.
8. Gould RL, Pazdro R. Impact of Supplementary Amino Acids, Micronutrients, and Overall Diet on Glutathione Homeostasis. Nutrients 2019;11:1056.
9. Sarıkaya E. Doğan S. Glutathione Peroxidase in Health and Diseases. In Glutathione Peroxidase in Health and Disease. IntechOpen 2020.
10. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione Metabolism and Its Implications for Health. J Nutr 2004;134:489-92.
11. El-Hafidi M, Franco M, Ramírez AR, Sosa JS, Flores JAP, Acosta OL, et al. Glycine Increases Insulin Sensitivity and Glutathione Biosynthesis and Protects against Oxidative Stress in a Model of Sucrose-Induced Insulin Resistance. Oxid Med Cell Longev 2018;2018:2101562.
12. Franco R, Cidlowski JA. Glutathione efflux and cell death. Antioxidants Redox Signal 2012;17:1694-713.
13. Fico A, Manganelli G, Cigliano L, Bergamo P, Abrescia P, Franceschi C, et al. 2-deoxy-d-ribose induces apoptosis by inhibiting the synthesis and increasing the efflux of glutathione. Free Radic Biol Med 2008;45:211-7.
14. Hammond CL, Marchan R, Krance SM, Ballatori N. Glutathione export during apoptosis requires functional multidrug resistance-associated proteins. J Biol Chem 2007;282:14337-47.
15. Laberge RM, Karwatsky J, Lincoln MC, Leimanis ML, Georges E. Modulation of GSH levels in ABCC1 expressing tumor cells triggers apoptosis through oxidative stress. Biochem Pharmacol 2007;73:1727-37.
16. Sodani K, Patel A, Kathawala RJ, Chen ZS. Multidrug resistance associated proteins in multidrug resistance. Chin J Cancer 2012;31:58-72.
17. Bozkurt E, Atmaca H, Uzunoğlu S. Multiple drug resistance in glioblastoma tumors. General Medical Journal 2013;23:133-43.

18. Boumendjel A, Boutonnat J, Robert J. ABC Transporters and Multidrug Resistance. John Wiley & Sons Inc, Hoboken, New Jersey; 2009.p.459.
19. Bloise E, Ortiga-Carvalho TM, Reis FM, Lye SJ, Gibb W, Matthews SG. ATP-binding cassette transporters in reproduction: a new frontier. Human reproduction update 2016;22:164-81.
20. Bársony O, Szalóki G, Türk D, Tarapcsák S, Gutay-Tóth Z, Bacsó Z,et al. A single active catalytic site is sufficient to promote transport in P-glycoprotein. Sci Rep 2016;6:24810.
21. Han LW, Gao C, Mao Q. An update on expression and function of P-gp/ABCB1 and BCRP/ABCG2 in the placenta and fetus. Expert opin drug metab toxicol 2018;14:817-29.
22. Kruh GD, Guo Y, Hopper-Borge E, Belinsky MG, Chen ZS. ABCC10, ABCC11, and ABCC12. Pflugers Arch 2007;453:675-84.
23. Gupta A, Shah K, Oza MJ, Behl T. Reactivation of p53 gene by MDM2 inhibitors: a novel therapy for cancer treatment. Biomed Pharmacother 2019;109:484-92.
24. Harati-Sadegh M, Kohan L, Teimoori B, Mehrabani M, Salimi S. Analysis of polymorphisms, promoter methylation, and mRNA expression profile of maternal and placental P53 and P21 genes in preeclamptic and normotensive pregnant women. J Biomed Sci 2019;26:92.
25. Kulesza M, Dansonka-Mieszkowska A, Pieńkowska-Grela B. Repair or perish - the role of p53 protein in a cell's life. Nowotwory Journal of Oncology 2019;69:168-78.
26. Stiewe T, Haran TE. How mutations shape p53 interactions with the genome to promote tumorigenesis and drug resistance. Drug Resist Updat 2018;38:27-43.
27. Ye Q, Liu K, Shen Q, Li Q, Hao J, Han F, et al. Reversal of Multidrug Resistance in Cancer by Multi-Functional Flavonoids. Front Oncol 2019;9:487.
28. Zhu L, Han MB, Gao Y, Wang H, Dai L, Wen Y, et al. Curcumin triggers apoptosis via upregulation of Bax/Bcl-2 ratio and caspase activation in SW872 human adipocytes. Mol Med Rep 2015;12:1151-6.
29. Karagül Mİ, Aktas S, Yetkin D, Bayrak G, Çelikcan D. P53, Bcl2 and Bax Expression and Apoptosis in Perifosine and Vitamin D-Treated Endometrial Cancer Cell Line (HEC1A). Proceedings 2018;2:1564.
30. Meichner K, Fogle JE, English L, Suter SE. Expression of Apoptosis-regulating Proteins Bcl-2 and Bax in Lymph Node Aspirates from Dogs with Lymphoma. J Vet Intern Med 2016;30:819-26.
31. Brauns SC, Dealtry G, Milne P, Naudé R, Van de Venter M. Caspase-3 activation and induction of PARP cleavage by cyclic dipeptide cyclo (Phe-Pro) in HT-29 cells. Anticancer Res 2005;25:4197-202.
32. Hou Z, Cui Y, Xing H, Mu X. Down-expression of poly(ADP-ribose) polymerase in p53-regulated pancreatic cancer cells. Oncol Lett 2018;15:1943-8.
33. Gupta SC, Sharma A, Mishra M, Mishra RK, Chowdhuri DK. Heat shock proteins in toxicology: How close and how far? Life Sci 2010;86:377-84.
34. Saibil H. Chaperone machines for protein folding, unfolding and disaggregation. Nat Rev Mol Cell Biol 2013;14:630-42.
35. Park CJ, Seo YS. Heat Shock Proteins: A Review of the Molecular Chaperones for Plant Immunity. Plant Pathol J 2015;31:323-33.
36. Ikwegbue PC, Masamba P, Oyinloye BE, Kappo AP. Roles of Heat Shock Proteins in Apoptosis, Oxidative Stress, Human Inflammatory Diseases, and Cancer. Pharmaceuticals (Basel) 2017;11:2.
37. Kennedy D, Jäger R, Mosser DD, Samali A. Regulation of apoptosis by heat shock proteins. IUBMB Life 2014;66:327-38.
38. Zhang X, Zhang L, Wang S, Wu D, Yang W. Decreased functional expression of Grp78 and Grp94 inhibits proliferation and attenuates apoptosis in a human gastric cancer cell line in vitro. Oncol Lett 2015;9:1181-6.
39. Lu T, Wang Y, Xu K, Zhou Z, Gong J, Zhang Y, et al. Co-downregulation of GRP78 and GRP94 induces apoptosis and inhibits migration in prostate cancer cells. Open Life Sci 2019;14:384-91.
40. 40. Tsai YL, Ha DP, Zhao H, Carlos AJ, Wei S, Pun TK, et al. Endoplasmic reticulum stress activates SRC, relocating chaperones to the cell surface where GRP78/CD109 blocks TGF-β signaling. Proc Natl Acad Sci U S A 2018;115:E4245-54.
41. Indran IR, Tufo G, Pervaiz S, Brenner C. Recent advances in apoptosis, mitochondria and drug resistance in cancer cells. Biochim Biophys Acta 2011;1807:735-45.
42. Aneja R, Liu M, Yates C, Gao J, Dong X, Zhou B, et al. Multidrug Resistance-Associated Protein-Overexpressing Teniposide-Resistant Human Lymphomas Undergo Apoptosis by a Tubulin-Binding Agent. Cancer Res 2008;68:1495-503.
43. Wesołowska O, Wiśniewski J, Sroda-Pomianek K, Bielawska-Pohl A, Paprocka M, Duś D, et al. Multidrug Resistance Reversal and Apoptosis Induction in Human Colon Cancer Cells by Some Flavonoids Present in Citrus Plants. J Nat Prod 2012;75:1896-902.
44. Mansoori B, Mohammadi A, Davudian S, Shirjang S, Baradaran B. The Different Mechanisms of Cancer Drug Resistance: A Brief Review. Adv Pharm Bull 2017;7:339-48.
45. Mesci S, Gül M, Yıldırım T. Antioxidant and cytotoxic activity studies of sulfur containing glycine imine derivatives MCF-7 and DLD-1 cell lines. Clin Exp Health Sci 2021;11:81-90.
46. Freshney RI. Culture of animal cells: a manual of basic technique and specialized applications. John Wiley & Sons; 2015.
47. Mates JM, Segura JA, Alonso FJ, Marquez J. Sulphur-containing non enzymatic antioxidants: therapeutic tools against cancer. Front Biosci (Schol Ed) 2012;4:722-48.
48. Estrela JM, Ortega A, Obrador E. Glutathione in cancer biology and therapy. Crit Rev Clin Lab Sci 2006;43:143-81.
49. Porta C, Moroni M, Guallini P, Torri C, Marzatico F. Antioxidant enzymatic system and free radicals pathway in two different human cancer cell lines. Anticancer Res 1996;16:2741-7.
50. Babich H, Ackerman NJ, Burekhovich F, Zuckerbraun HL, Schuck AG. Gingko biloba leaf extract induces oxidative stress in carcinoma HSC-2 cells. Toxicol In Vitro 2009;23:992-9.
51. Han YH, Park WH. The effects of N-acetyl cysteine, buthionine sulfoximine, diethyldithiocarbamate or 3-amino-1,2,4-triazole on antimycin A-treated Calu-6 lung cells in relation to cell growth, reactive oxygen species and glutathione. Oncol Rep 2009;22:385-91.

52. 52. Niture SK, Velu CS, Smith QR, Bhat GJ, Srivenugopal KS. Increased expression of the MGMT repair protein mediated by cysteine prodrugs and chemopreventative natural products in human lymphocytes and tumor cell lines. Carcinogenesis 2007;28:378-89.

53. Rosado JO, Salvador M, Bonatto D. Importance of the transsulfuration pathway in cancer prevention and promotion. Mol Cell Biochem 2007;301:1-12.

54. Domenicotti C, Marengo B, Verzola D, Garibotto G, Traverso N, Patriarca S, et al. Role of PKC-delta activity in glutathione-depleted neuroblastoma cells. Free Radic Biol Med 2003;35:504-16.