

## Sensitization Effects of Repurposed Blood Pressure-regulating Drugs on Drug-resistant Cancer Cells

CHUNXUE JIANG\*, TIAN ZHENG\*, JAE HYEON PARK, JIN-SOL LEE,  
YUNMOON OH, AMIT KUNDU, HYUNG SIK KIM and SUNGPIL YOON

*School of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea*

**Abstract.** *Background/Aim:* We investigated drugs that could sensitize KBV20C cancer cells resistant to eribulin or vincristine (VIC) treatment and assessed their associated mechanisms of action. *Materials and Methods:* Such cancer cells were known to overexpress P-glycoprotein (P-gp). Considering that reserpine (P-gp inhibitor) plays a regulatory role in patients with high blood pressure, we investigated the effect of low doses of 27 blood pressure-regulating drugs on VIC-resistant KBV20C cells. This was done to identify drugs that could be repurposed for sensitizing antimitotic drug-resistant KBV20C cells at relatively low doses. Fluorescence-activated cell sorting (FACS), annexin V analyses, rhodamine uptake tests and western-blot analysis were performed to further investigate the mechanism of action of such drugs. *Results:* We found that co-treatment with amiodarone, nicardipine, carvedilol, or vardenafil at low doses could highly sensitize KBV20C cells treated with eribulin or VIC. These drugs reduced cellular viability, increased G<sub>2</sub> arrest and up-regulated apoptosis when co-administered with eribulin or VIC. Considering that they sensitize with either co-treatment of eribulin or VIC, we assumed that they can be combined with other antimitotic drugs to sensitize the resistant cancer cells. Through detailed quantitative analysis, we found that eribulin with amiodarone had a higher sensitization effect than eribulin with nicardipine or eribulin with carvedilol. We found that reserpine had the highest P-gp-inhibitory activity, indicating that eribulin- or VIC-

*reserpine sensitization involves the P-gp inhibitory effects of reserpine. However, we found that amiodarone, nicardipine, carvedilol and vardenafil had very low P-gp inhibitory activity. Moreover, we found that cells co-treated with VIC-carvedilol down-regulated expression of pERK. Conclusion:* Highly antimitotic drug-resistant KBV20C cells can be sensitized by co-treatment with the repurposed blood pressure-regulating drugs amiodarone, nicardipine, carvedilol or vardenafil. These findings indicate that the repurposed blood pressure-regulating drugs may potentially be used in drug-resistant cancer patients without any toxic effects due to P-gp inhibition.

Antimitotic drugs, such as paclitaxel, docetaxel, vincristine (VIC), vinorelbine, vinblastine and eribulin inhibit mitosis by targeting microtubules and preventing their polymerization or depolymerization (1-3). Although antimitotic drugs are widely used to treat cancer, cancer cells can develop resistance to these drugs in various ways. P-glycoprotein (P-gp) overexpression is a well-known mechanism of resistance to these drugs. P-gp is a membrane channel that can expel antimitotic drugs, thereby avoiding drug-induced toxicity (4, 5). Identifying mechanisms for sensitizing cancer cells that overexpress P-gp can lead to better treatment of patients who develop resistance to these drugs. Although P-gp inhibitors have been developed, their toxicity in normal cells leads to treatment failure. Therefore, it is important to investigate novel therapeutic options without P-gp inhibition for drug-resistant cancer cells overexpressing P-gp.

In this study, we evaluated novel repurposed drugs for their sensitizing efficacy in drug-resistant cancer cells overexpressing P-gp when used in combination with antimitotic drugs. In addition, we investigated the mechanisms involved in this process. The urgent need for pharmacological treatments for drug-resistant cancer cells can be effectively addressed with novel mechanisms of action for repurposed drugs that can be used without further toxicity evaluation (6-8).

\*These Authors contributed equally to this work.

*Correspondence to:* Sunpil Yoon, Ph.D., School of Pharmacy, Sungkyunkwan University, 2066 Seobu-ro, Jangan-gu, Suwon, Gyeonggi-do, 16419, Republic of Korea. Tel: +82 1055024893, Fax: +82 312928800, e-mail: syoon88@gmail.com

**Key Words:** Reserpine, amiodarone, nicardipine, carvedilol, vardenafil, blood pressure-related drugs, repositioning drug, resistant cancer, P-gp.

Previous studies have shown that reserpine, a drug that regulates high blood pressure, acts as an inhibitor of P-gp in cancer cells and exhibits drug-sensitization effects in drug-resistant cancer cells overexpressing P-gp (9, 10). In addition, drugs that regulate blood pressure have been shown to be correlated with P-gp-inhibitory activities (11-17). These drugs have also been found to sensitize drug-resistant cancer cells (11-17). However, the mechanisms of action of individual blood pressure-regulating drugs have not yet been investigated.

In this study, we investigated different drugs that regulate blood pressure for their sensitizing effects in drug-resistant cancer cells. Based on the existing literature, we identified the 27 drugs that regulate blood pressure. We then identified individual drugs with relatively low half-maximal inhibitory concentrations to sensitize drug-resistant KBV20C cancer cells overexpressing P-gp. We also investigated the mechanisms involved in the sensitization of drug-resistant cancer cells. We found that low doses of amiodarone, nicardipine, carvedilol, and vardenafil can sensitize antimitotic drug-resistant KBV20C cells. As these drugs are used in clinical settings as drugs for blood pressure disorders, these results can contribute to the development of therapies based on drugs regulating blood pressure in the co-treatment of highly drug-resistant tumors.

## Materials and Methods

**Reagents and cell culture.** Rhodamine123 (Rhodamine) and verapamil were obtained from Sigma-Aldrich (St. Louis, MO, USA). VIC and vinblastine were obtained from Enzo Life Sciences (Farmingdale, NY, USA). Reserpine, amiodarone, nicardipine, propafenone, carvedilol, amlodipine, diltiazem, nifedipine, nimodipine, doxazosin mesylate, triamterene, isradipine, midodrine, quinidine, prazosin, ethacrynic acid, losartan potassium, benazepril, eplerenone, labetalol, methyclothiazide, metolazone, valsartan, telmisartan, spironolactone, disopyramide, dipyridamole, and vardenafil were purchased from Selleckchem (Houston, TX, USA). Aqueous solutions of eribulin (Eisai Korea, Seoul, Republic of Korea) were obtained from the National Cancer Center in Republic of Korea.

The KBV20C (resistant type) and KB (sensitive parent type) are human oral squamous carcinoma cell lines; they were generously gifted from Dr. Yong Kee Kim (College of Pharmacy, Sookmyung Women's University, Seoul, Republic of Korea) and have been previously studied (18-20). All cell lines were grown in RPMI 1640 containing 10% fetal bovine serum, 100 U/ml penicillin and 100 µg/ml streptomycin (WelGENE, Daegu, Republic of Korea).

**Microscopic observations.** The effect of drugs on cell density (cell growth) was observed and compared with control group according to microscopic observations, as previously described (21-23). The cells were treated with the 5 µM blood-pressure regulating drugs or 0.1% DMSO (Control), alone and in combination with eribulin or vincristine for 24 h. The cells were then examined in two independent experiments using an ECLIPSETs2 inverted routine microscope (Nikon, Tokyo, Japan) with a 40× or 100× objective lens.

**Cell viability assay.** The EZ-CyTox cell viability assay kit (Daeillab, Republic of Korea) was used for measuring cellular proliferation, as previously described (21-23). Briefly, the cells were treated with the 5-10 µM blood-pressure regulating drugs, 10 µM of verapamil or 0.1% DMSO (Control), alone and in combination with eribulin or vincristine for 48 hours. The cells were then treated with EZ-CyTox solution for 1 h at 37°C. The absorbance at 450 nm was measured using the VERSA MAX Microplate Reader (Molecular Devices Corp., Sunnyvale, CA, USA). All experiments were performed in triplicate and repeated twice.

**Fluorescence-activated cell sorting (FACS) analysis.** FACS analysis was performed as previously described (24-26). The cells were treated with the 5 µM blood-pressure regulating drugs, 10 µM of verapamil or 0.1% DMSO (Control), alone and in combination with eribulin or vincristine for 24 h. The stained cells with propidium iodide (PI) staining solution (100 µg/ml RNase A and 50 µg/ml PI) were quantified in two independent experiments for relative DNA content using a Guava EasyCyte Plus Flow Cytometer (Merck Millipore, Burlington, MA, USA).

**Annexin V analysis.** Annexin V analysis was conducted using the annexin V-fluorescein isothiocyanate (FITC) staining kit (BD Bioscience, Franklin, NJ, USA) as previously described (24-26). The cells were treated with the 5 µM blood-pressure regulating drugs or 0.1% DMSO (Control), alone and in combination with eribulin or vincristine for 24 h. The stained cells in Annexin V-FITC and PI solution were analyzed in two independent experiments using a Guava EasyCyte Plus Flow Cytometer (Merck Millipore, Burlington, MA, USA).

**Rhodamine uptake tests.** The tests used to assess the ability of a drug to inhibit P-gp were based on a previously described method (26-28). Briefly, cells were treated with 5 µM amiodarone, 5 µM nicardipine, 5 µM carvedilol, 5 µM vardenafil, or 5 µM reserpine and incubated for 24 hours at 37°C. The cells were subsequently incubated with 2 µg/ml rhodamine for 60 min at 37°C. The stained cells were analyzed in two independent experiments using a Guava EasyCyte Plus Flow Cytometer (Merck Millipore, Burlington, MA, USA).

**Western blot analysis.** Briefly, cells were treated with 5 nM vincristine, 5 µM carvedilol, 5 nM VIC with 5 µM carvedilol or 0.1% DMSO (control). After 24 h, PRO-PREP™ protein extract solution (iNtRON, Seongnam, Republic of Korea) was used for isolating total cellular proteins as previously described (26-28). A protein assay kit (Bio-Rad, Hercules, CA, USA) was used for measuring total protein concentration. The proteins were then subjected to western blot analysis as previously described (24, 28, 29).

Antibodies against P-gp (517312, 1:1,000, dilution) were obtained from Calbiochem (Merck Millipore, USA). Antibodies against pAkt (#9271, 1:1,000, dilution), PI3K (#4292, 1:1,000, dilution), pPI3K (#4228, 1:2,000, dilution), mTOR (#2983, 1:1,000, dilution), pmTOR (#2971, 1:1,000, dilution), Erk (#4695, 1:1,000, dilution), pErk (#4370, 1:500, dilution), p38 (#9212, 1:1,000, dilution), pp38 (#9211, 1:1,000, dilution), Jnk (#9252, 1:1,000, dilution), pJnk (#9251, 1:1,000, dilution), NFκB (#3045, 1:1,000, dilution), pNFκB (#3033, 1:1,000, dilution), and GAPDH (#5174, 1:1,000, dilution) were obtained from Cell Signaling Technology (Danvers, MA, USA). Antibodies against Akt-1 (sc-5298, 1:1,000, dilution) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

**RT-PCR.** Total RNA was extracted and quantified as previously described (30, 31). Briefly, total RNA from cells grown in 60 mm dishes was extracted using Qiazol Lysis Reagent (Qiazen, Germantown, MD, USA) according to the manufacturer's instructions and stored at  $-80^{\circ}\text{C}$  until use. The purity and quantity of the extracted total RNA were determined using a spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA) at 260 and 280 nm. Two micrograms of total RNA from each sample was synthesized as cDNA *via* reverse transcription using the Reverse Transcription Master Premix (Elpis Biotech, Daejeon, Republic of Korea).

Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using the FastStart Essential DNA Green Master (Roche, Penzberg, Germany) in a LightCycler 96 Real-Time PCR system (Roche, Penzberg, Germany). The PCR primers used were 5'-CCCATCATGTGCAATAGCAGG-3' (forward) and 5'-GTTCAAACCTCTGCTCCTGA-3' (reverse) for the P-gp/ABCB1 gene and 5'-CGGAGTCAACGGATTGGTCGTAT-3' (forward) and 5'-AGCCTTCTCCATGGTGGTGAAGAC-3' (reverse) for GAPDH. The relative fold change in gene expression was calculated using the  $2^{-\Delta\Delta C_t}$  method (30, 31).

**Statistical analysis.** All data are presented as the mean $\pm$ S.D. from at least three independent experiments. Statistical analysis was performed using one-way analysis of variance, ANOVA followed by Bonferroni's test. For RT-PCR, statistical analysis was performed by using Student's *t*-test. Analysis was performed using GraphPad Prism software (version 5.0; GraphPad Software, CA, USA). Statistical significance was set at  $p < 0.05$ .

## Results

**Five drugs sensitize eribulin-treated drug-resistant KBV20C cancer cells at low doses.** We aimed to identify repurposed drugs that sensitize drug-resistant cancer cells overexpressing P-gp to treatments with chemotherapeutic drugs. Previously, the high blood-pressure drug reserpine inhibits P-gp in cancer cells and sensitizes P-gp overexpressing drug-resistant cancer cells (9, 10). In addition, it has been known that blood pressure regulating drugs correlate with P-gp-inhibitory activity (11-17). However, comparisons of the individual blood-pressure regulating drugs and their exact mechanisms of action have not been investigated in detail. In this study, we focused on blood-pressure regulating drugs that sensitize P-gp-overexpressing resistant cancer cells at low doses and investigated their mechanism of sensitization. The KBV20C cell line is a very useful model of highly eribulin-resistant cancer cells. We previously showed that the concentration of eribulin required for the induction of a similar rate of apoptosis was approximately 500-fold higher than that required by the parental drug-sensitive KB cell line (20, 32). We assume that additional findings for repurposed drugs regulating blood pressure can increase potential applications in personalized medicine. Therefore, we performed detailed analysis with 27 known drugs regulating blood pressure, including amiodarone, nicardipine, propafenone, carvedilol, amlodipine, diltiazem, nifedipine, nimodipine, doxazosin

mesylate, triamterene, isradipine, midodrine, quinidine, prazosin, ethacrynic acid, losartan potassium, benazepril, eplerenone, labetalol, methyclothiazide, metolazone, valsartan, telmisartan, spironolactone, disopyramide, dipyridamole and vardenafil.

We compared the sensitizing effects of these blood-pressure regulating drugs to reserpine (positive control), which is a high P-gp inhibitor. As seen in Figure 1A, reserpine was also shown to sensitize eribulin-treated KBV20C cells. We did not detect sensitization effects for co-treatment of reserpine in drug-sensitive parental KB cells (data not shown), suggesting that P-gp inhibitory activity of reserpine is specifically increasing sensitization for drug-resistant KBV20C cells overexpressing P-gp.

First, we performed a quantitative analysis with a cell viability test. As seen in Figure 1B-E, five drugs (amiodarone, nicardipine, carvedilol, quinidine, and vardenafil) showed highly reduced viability in eribulin-treated KBV20C cells, with eribulin co-treatments leading to cultures with  $>40\%$  viability compared to the control. A lower dose of the five drugs is sufficient and as effective as reserpine in sensitizing drug-resistant cancer cells overexpressing P-gp, although treatment with an equivalent dose of reserpine (as a positive control) produced the highest sensitization effects on cells co-treated with eribulin (Figure 1B-E). There was no difference in viability between the control cells and those receiving individual treatment with the drugs regulating blood pressure (Figure 2A), suggesting that sensitization by eribulin co-treatments of the five drugs regulating blood pressure resulted in synergistic effects in the eribulin-resistant cancer cells.

**Amiodarone, nicardipine, carvedilol and vardenafil result in higher sensitization of eribulin-treated drug-resistant cancer cells.** It is important to identify which drugs among those regulating blood pressure produce better sensitization effects at low doses. Further, both cellular viability and cell density with lower concentrations showed these four drugs (amiodarone, nicardipine, carvedilol, and vardenafil) have higher sensitization effects compared to others (data not shown). Further detailed viability analysis with lower concentrations of the four identified drugs were performed to identify the most effective in the eribulin-treated drug-resistant KBV20C cells. As seen in Figure 2B and C, amiodarone at a low dose has much higher sensitization than other drugs for eribulin-treated KBV20C cells.

In summary, we observed that 4 of the 27 known blood-pressure drugs regulating blood pressure produced sensitization effects (reduced cell viability with eribulin co-treatment) at low doses. Based on this observation, we concluded that the four drugs (amiodarone, nicardipine, carvedilol and vardenafil) can be used to reduce drug toxicity and sensitize eribulin-resistant cancer cells.

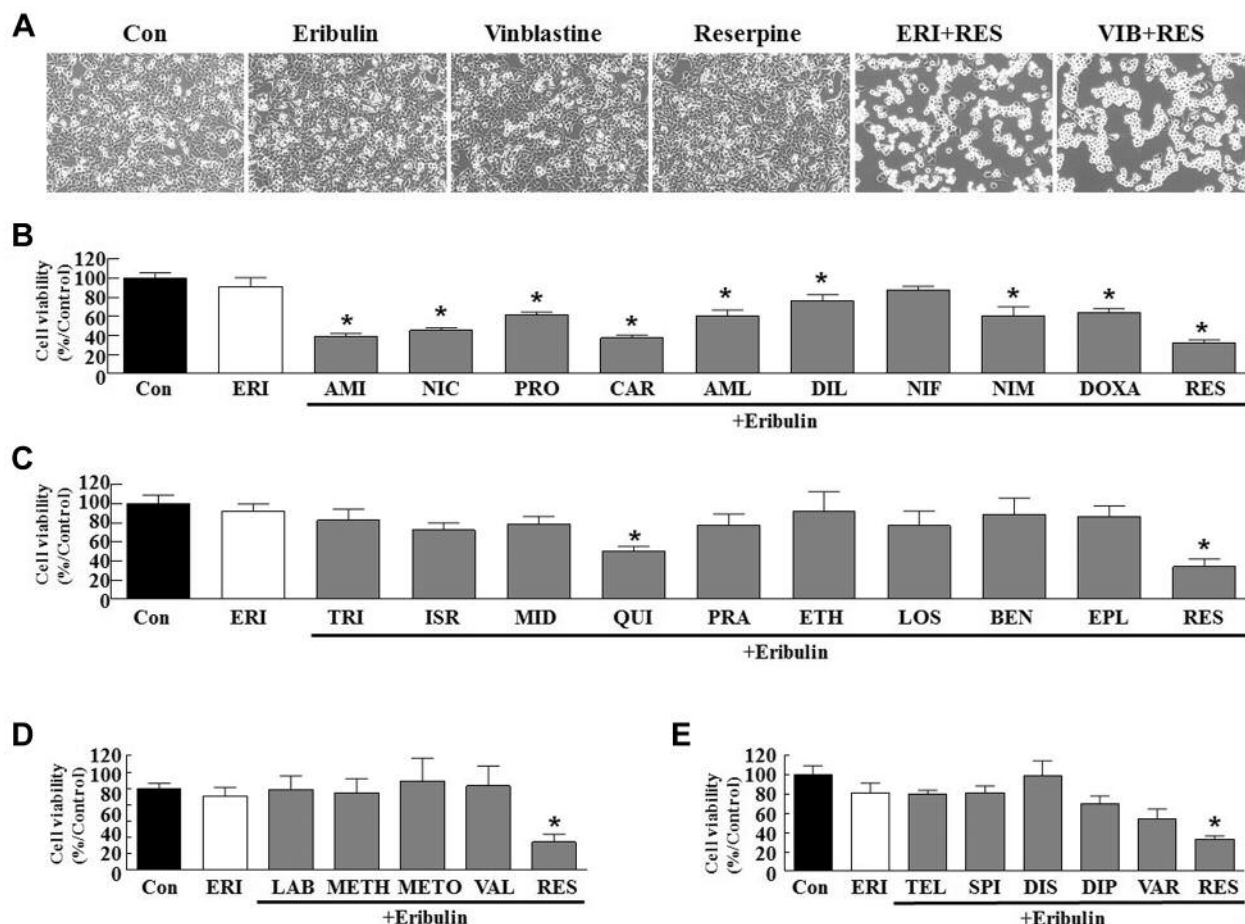
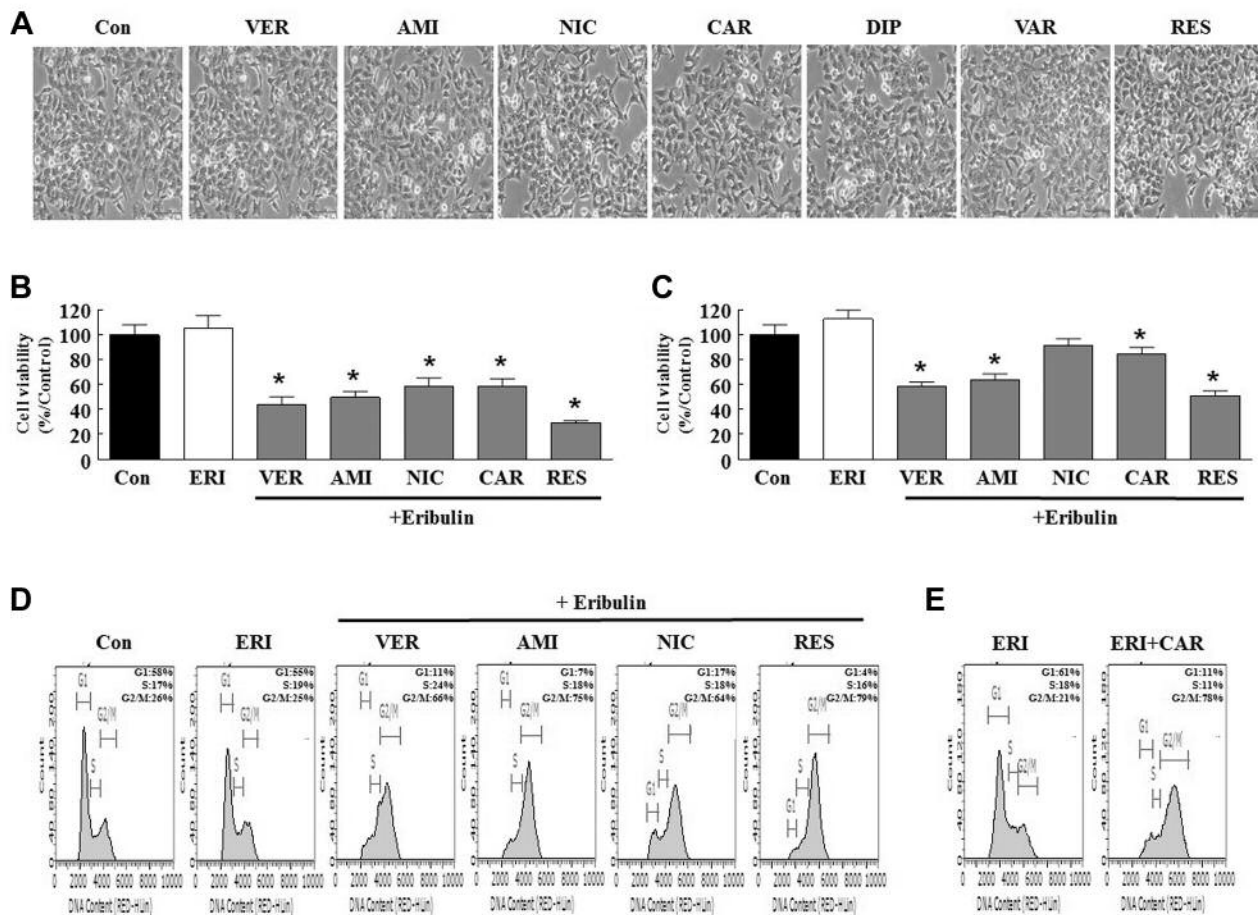


Figure 1. Five drugs result in higher sensitization of drug-resistant KBV20C cancer cells treated with eribulin. (A) KBV20C cells were grown on 60 mm-diameter dishes and treated with 10 nM eribulin, 5 nM vinblastine, 5  $\mu$ M reserpine, 10 nM eribulin with 5  $\mu$ M reserpine (ERI+RES), 5 nM vinblastine with 5  $\mu$ M reserpine (VIB+RES), or 0.1% DMSO (control). After 1 day, all cells were observed using an inverted microscope at  $\times 100$  magnification. (B-E) Cell viability assay was performed as described in Materials and Methods. The data are presented as the mean  $\pm$  SD of at least two experiments repeated in triplicate. Significantly different at  $*p < 0.05$  compared to the corresponding control. (B) KBV20C cells were plated on 96-well plates and grown to 30%-40% confluence. The cells were then stimulated for 48 h with 10  $\mu$ M of amiodarone (AMI), nicardipine (NIC), propafenone (PRO), carvedilol (CAR), amlodipine (AML), diltiazem (DIL), nifedipine (NIF), nimodipine (NIM), doxazosin mesylate (DOXA), and reserpine (RES) and in combination with 10 nM eribulin or alone, or 0.1% DMSO (control). (C) KBV20C cells were plated on 96-well plates and grown to 30%-40% confluence. The cells were then stimulated for 48 h with 10  $\mu$ M of triamterene (TRI), isradipine (ISR), midodrine (MID), quinidine (QUI), prazosin (PRA), ethacrynic acid (ETH), losartan potassium (LOS), benazepril (BEN), eplerenone (EPL), and reserpine (RES) and in combination with 10 nM eribulin or alone, or 0.1% DMSO (control). (D) KBV20C cells were plated on 96-well plates and grown to 30%-40% confluence. The cells were then stimulated for 48 h with 10  $\mu$ M of labetalol (LAB), methyclothiazide (METH), metolazone (METO), valsartan (VAL), and reserpine (RES) and in combination with 10 nM eribulin or alone, or 0.1% DMSO (control). (E) KBV20C cells were plated on 96-well plates and grown to 30%-40% confluence. The cells were then stimulated for 48 h with 10  $\mu$ M of telmisartan (TEL), spironolactone (SPI) disopyramide (DIS), dipyrindamole (DIP), vardenafil (VAR), and reserpine (RES) and in combination with 10 nM eribulin or alone, or 0.1% DMSO (control).

Co-treatment with selected blood-pressure regulating drugs increased  $G_2$  arrest in eribulin-treated KBV20C cells similar to verapamil treatment. Next, the sensitizing effects of verapamil were compared to the selected blood-pressure regulating drugs on eribulin-treated KBV20C cells. As shown in Figure 2B, 5  $\mu$ M of the selected blood-pressure regulating drugs (amiodarone, nicardipine, or carvedilol) and

10  $\mu$ M of verapamil produced similar sensitization effects on cells treated with eribulin, suggesting that a low dose of amiodarone, nicardipine or carvedilol is sufficient and as effective as the P-gp inhibitor verapamil, in sensitizing drug-resistant cancer cells overexpressing P-gp.

To further clarify the mechanism of action for co-treatment combining eribulin and the identified blood-pressure



**Figure 2.** Co-treatment with the selected four blood-pressure regulating drugs (amiodarone, nicardipine, carvedilol, and verapamil) increased  $G_2$  arrest in KBV20C cells treated with eribulin, similar to treatment with verapamil. (A) KBV20C cells were grown on 60 mm-diameter dishes and treated with 10  $\mu$ M of verapamil (VER), amiodarone (AMI), nicardipine (NIC), carvedilol (CAR), dipyridamole (DIP), vardenafil (VAR), and reserpine (RES), or 0.1% DMSO (CON). After 1 day, all cells were observed using an inverted microscope at  $\times 100$  magnification. (B) KBV20C cells were plated on 96-well plates and grown to 30%-40% confluence. The cells were then stimulated for 48 h with 10  $\mu$ M of verapamil (VER), 5  $\mu$ M of amiodarone (AMI), 5  $\mu$ M of nicardipine (NIC), 5  $\mu$ M of carvedilol (CAR), 5  $\mu$ M of reserpine (RES) and in combination with 10 nM eribulin or alone, or 0.1% DMSO (Con). Cell viability assay was performed as described in Materials and Methods. The data are presented as the mean $\pm$ SD of at least two experiments repeated in triplicate. Significantly different at  $*p<0.05$  compared to the corresponding control. (C) KBV20C cells were plated on 96-well plates and grown to 30%-40% confluence. The cells were then stimulated for 48 h with 5  $\mu$ M of verapamil (VER), 2.5  $\mu$ M of amiodarone (AMI), 2.5  $\mu$ M of nicardipine (NIC), 2.5  $\mu$ M of carvedilol (CAR), 2.5  $\mu$ M of reserpine (RES) and in combination with 10 nM eribulin or alone, or 0.1% DMSO (Con). Cell viability assay was performed as described in Materials and Methods. The data are presented as the mean $\pm$ SD of at least two experiments repeated in triplicate. Significantly different at  $*p<0.05$  compared to the corresponding control. (D, E) KBV20C cells were grown on 60 mm-diameter dishes and treated with 10 nM eribulin (ERI), 10 nM eribulin with 10  $\mu$ M verapamil (VER), 10 nM eribulin with 5  $\mu$ M amiodarone (AMI), 10 nM eribulin with 5  $\mu$ M nicardipine (NIC), 10 nM eribulin with 5  $\mu$ M carvedilol (CAR), or 0.1% DMSO (Con). After 24 h, FACS analyses were performed as described in Materials and Methods.

regulating drugs, we performed fluorescence-activated cell sorting (FACS) analyses. As shown in Figure 2D and E, eribulin-amiodarone, eribulin-nicardipine and eribulin-carvedilol co-treatments considerably increased the number of cells in  $G_2$  arrest compared to control cells. Treatment with 5  $\mu$ M reserpine or 10  $\mu$ M verapamil (as positive controls) also produced the higher sensitization effects on cells co-

treated with eribulin (Figure 2D). This indicates that the reduction in cellular viability resulted from cell cycle arrest. The results confirmed that amiodarone, nicardipine or carvedilol is as effective as verapamil or reserpine in sensitizing eribulin-resistant cancer cells. It also suggests that amiodarone, nicardipine or carvedilol can be used at a low dose with reduced drug toxicity to sensitize eribulin-resistant

cancer cells. Altogether, these data indicate that co-treatment with the selected blood-pressure regulating drugs sensitized KBV20C cells to eribulin treatment, with similar efficacy to that of verapamil in both viability and G<sub>2</sub> arrest.

*Low doses of amiodarone, nicardipine, carvedilol and vardenafil also increase the sensitization of KBV20C cells treated with other anti-mitotic drugs.* We also investigated whether the selected blood-pressure regulating drugs were effective in combination with other anti-mitotic drugs. We tested sensitization with VIC, an anti-mitotic drug that is routinely used as a chemotherapeutic agent in cancer (33-35). Previously, we showed that KBV20C-resistant cancer cells present with a VIC-resistant phenotype through P-gp overexpression (18, 36).

As seen in microscopic observations shown in Figure 3B, 5  $\mu$ M of amiodarone, nicardipine, carvedilol, or vardenafil produce similar sensitizing effects when combined with VIC. As we observed in eribulin with reserpine (positive control) in sensitizing P-gp-overexpressing resistant cancer cells (Figure 1B-E), reserpine also produced the highest sensitization effects on cells co-treated with VIC (Figure 3A and B).

In a detailed quantitative viability analysis, we confirmed that low dose of the four drugs (amiodarone, nicardipine, carvedilol, or vardenafil) is sufficient and as effective as reserpine in sensitizing drug-resistant cancer cells overexpressing P-gp (Figure 3A). But as observed in eribulin co-treatments (Figure 1C and E), VIC-quinidine and VIC-telmisartan did not increase sensitization-effects (Figure 3A and B).

This finding suggests that low doses of amiodarone, nicardipine, carvedilol or vardenafil could be combined with other anti-mitotic drugs to sensitize cancer cells overexpressing P-gp. In this regard, amiodarone, nicardipine, carvedilol or vardenafil could be applied to various drug-resistant cancer patients.

*Amiodarone, nicardipine, carvedilol and vardenafil strongly induce early apoptosis in VIC-treated drug-resistant KBV20C cells.* To further clarify the mechanism of action of the selected blood-pressure regulating drugs-VIC co-treatments, apoptotic analysis using annexin V staining was performed.

As seen in Figure 3C, a lower dose of amiodarone, nicardipine, carvedilol or vardenafil is sufficient and as effective as reserpine in sensitizing drug-resistant cancer cells overexpressing P-gp, although treatment with an equivalent dose of reserpine (as a positive control) produced the highest apoptosis on cells co-treated with VIC. The results also demonstrated that amiodarone, nicardipine, carvedilol or vardenafil has a similar sensitization effect for apoptosis. But, as we observed in both microscopic observation and viability assays (Figure 3A and B), VIC-quinidine has the lowest increase in apoptosis (Figure 3C). When the proportion of

apoptotic cells (in both early and late phases) was quantitatively estimated, we found that the proportions of the early apoptotic cells were approximately 2-fold higher than late apoptotic cells (Figure 3C). These results suggest that early apoptotic induction results in the sensitization effects of the blood-pressure regulating drugs-VIC co-treatments.

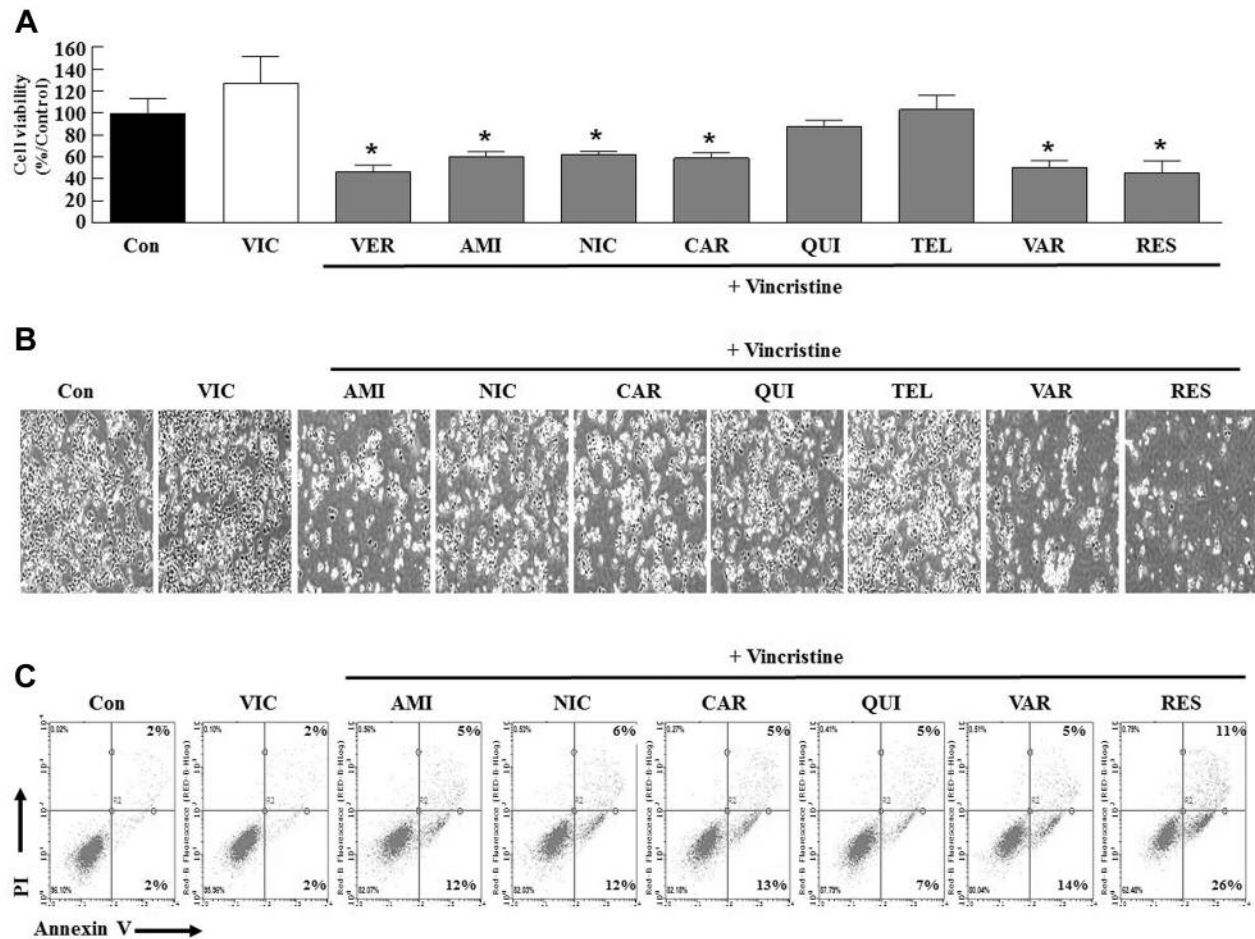
Overall, we demonstrated that among the 27 blood-pressure regulating drugs tested, amiodarone, nicardipine, carvedilol or vardenafil co-treatment led to high sensitization of drug-resistant KBV20C cells treated with eribulin or VIC, via both G<sub>2</sub> cell-cycle arrest and early apoptosis. Considering that these drugs sensitize KBV20C cells treated with eribulin or VIC at low doses, they may be useful in clinical settings, given these minimal toxic concentrations in normal cells.

*Four blood-pressure regulating drugs have low P-gp inhibitory activity in contrast to reserpine.* In the next phase of our investigation, we tested whether amiodarone, nicardipine, carvedilol, vardenafil and reserpine (as a positive control) increased the inhibition of the P-gp substrate efflux in P-gp overexpressing KBV20C cells since these drugs have drug-sensitization effects (26, 28). Rhodamine 123, a well-known P-gp substrate, was used to measure P-gp inhibition (24, 26, 28). In this experiment, yellow fluorescence in the cell was indicative of intracellular accumulation of rhodamine 123.

As shown in Figure 4A, reserpine showed the highest P-gp inhibitory activity, whereas the other blood-pressure regulating drugs had much lower activities. This suggests that P-gp inhibition by reserpine plays a key role in the highest sensitization by eribulin- or VIC-reserpine co-treatment. However, P-gp inhibition by amiodarone, nicardipine, carvedilol, vardenafil was much lower than that by reserpine, being slightly higher than (or similar to) control levels (Figure 4A). Considering that even with low P-gp inhibitory activity, amiodarone, nicardipine, carvedilol, vardenafil still sensitized KBV20C cells treated with eribulin or VIC, they might be useful in clinical settings due to minimal toxic P-gp inhibitory effects in normal cells.

We also observed whether amiodarone, nicardipine or carvedilol reduces P-gp mRNA levels in drug-resistant KBV20C cells overexpressing P-gp. As seen in Figure 4B, carvedilol reduced about 30% P-gp mRNA levels, whereas nicardipine did not change P-gp mRNA levels. Furthermore, we investigated whether carvedilol or VIC-carvedilol reduces P-gp protein levels. As seen in Figure 4C, we did not observe detectable reductions of P-gp in either carvedilol single treatment or VIC-carvedilol co-treatment. These results also suggest that P-gp mRNA or protein levels by amiodarone, nicardipine or carvedilol do not contribute much to sensitization effects in KBV20C cells treated with eribulin or VIC.

*Carvedilol reduces ERK pathway in drug-resistant KBV20C cells treated with VIC.* To further investigate the expression



**Figure 3.** Amiodarone, nicardipine, carvedilol, and vardenafil strongly induce early apoptosis in drug-resistant KBV20C cells treated with VIC. (A) KBV20C cells were plated on 96-well plates and grown to 30%-40% confluence. The cells were then stimulated for 48 h with 5  $\mu$ M of amiodarone (AMI), 5  $\mu$ M of nicardipine (NIC), 5  $\mu$ M of carvedilol (CAR), 5  $\mu$ M of quinidine (QUI), 5  $\mu$ M of telmisartan (TEL), 5  $\mu$ M of vardenafil (VAR), 5  $\mu$ M of reserpine (RES) and in combination with 5 nM vincristine (VIC) or alone, or 0.1% DMSO (Con). Cell viability assay was performed as described in Materials and Methods. The data are presented as the mean  $\pm$  SD of at least two experiments repeated in triplicate. Significantly different at  $*p < 0.05$  compared to the corresponding control. (B) KBV20C cells were grown on 60 mm-diameter dishes and treated with 24 h with 5  $\mu$ M of reserpine (RES), amiodarone (AMI), nicardipine (NIC), carvedilol (CAR), quinidine (QUI), telmisartan (TEL), and vardenafil (VAR) and in combination with 5 nM vincristine (VIC) or alone, or 0.1% DMSO (Con). After 1 day, all cells were observed using an inverted microscope at  $\times 40$  magnification. (C) KBV20C cells were grown on 60 mm-diameter dishes and treated with 5 nM vincristine (VIC), 5 nM vincristine with 5  $\mu$ M amiodarone (AMI), 5 nM vincristine with 5  $\mu$ M nicardipine (NIC), 5 nM vincristine with 5  $\mu$ M quinidine (QUI), 5 nM vincristine with 5  $\mu$ M of vardenafil (VAR), 5 nM vincristine with 5  $\mu$ M reserpine (RES), or 0.1% DMSO (Con). After 24 h, annexin V analyses were performed as described in Materials and Methods.

of proteins involved in cellular signaling pathways in VIC-carvedilol co-treatment (24, 27, 29, 37), western blot analysis was performed. As shown in Figure 4C and D, there were no significant differences in the expression of major signaling related proteins such as phosphate forms of Akt, PI3K, mTOR, p38, Jnk and NF $\kappa$ B between VIC-carvedilol and single treatments. Importantly, phosphorylated ERK levels were largely reduced following co-treatments, suggesting that it may increase sensitization-effect in VIC-carvedilol co-treatment. Considering that ERK activation is involved in

positive signals for cancer growth, reduced ERK pathway by VIC-carvedilol contributes to increased apoptosis of drug-resistant KBV20C cells *via* G<sub>2</sub> cell cycle arrest in drug-resistant KBV20C cells overexpressing P-gp.

Altogether, when we analyzed 27 known blood-pressure regulating drugs to identify sensitization drug-resistant cancer cells overexpressing P-gp, we could observe that amiodarone, nicardipine, carvedilol and vardenafil at low doses have higher sensitization effects than the other drugs. Therefore, they can be used to reduce drug toxicity and effectively sensitize drug-

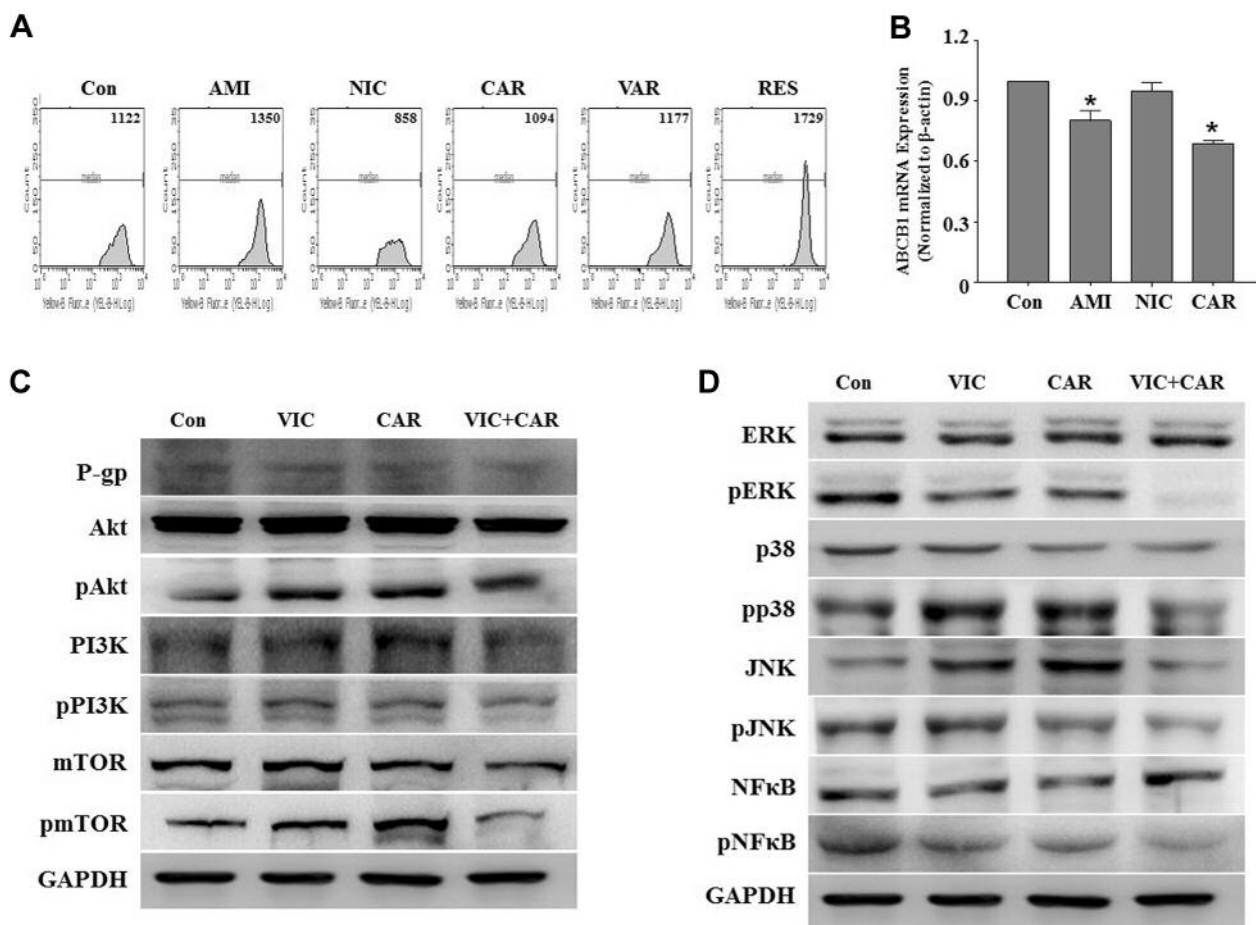


Figure 4. Four blood-pressure regulating drugs have low P-gp-inhibitory activity, and carvedilol reduces ERK pathway in drug-resistant KBV20C cells treated with VIC. (A) KBV20C cells were grown on 60 mm-diameter dishes and treated 10  $\mu$ M of amiodarone (AMI), nicardipine (NIC), carvedilol (CAR), vardenafil (VAR), and reserpine (RES), or 0.1% DMSO (Con). After 24 h, all cells were stained with rhodamine and examined by using FACS analysis, as described in Materials and Methods. (B) KBV20C cells were grown on 60 mm-diameter dishes and treated 10  $\mu$ M of amiodarone (AMI), nicardipine (NIC), and carvedilol (CAR), or 0.1% DMSO (Con). After 24 h, P-gp mRNA levels were performed as described in Materials and Methods. We performed Student's t-test to compare control and drugs-treated groups. Significantly different at \*p < 0.05 compared to the corresponding control. (C) KBV20C cells were plated on 60 mm-diameter dishes and treated with 5 nM vincristine (VIC), 5  $\mu$ M carvedilol (CAR), 5 nM VIC with 5  $\mu$ M carvedilol (VIC+CAR), or 0.1% DMSO (Con). After 24 h, western blot analysis was performed using antibodies against P-gp, Akt, pAkt, PI3K, pPI3K, mTOR, pmTOR, and GAPDH. (D) KBV20C cells were plated on 60 mm-diameter dishes and treated with 5 nM vincristine (VIC), 5  $\mu$ M carvedilol (CAR), 5 nM VIC with 5  $\mu$ M carvedilol (VIC+CAR), or 0.1% DMSO (Con). After 24 h, western blot analysis was performed using antibodies against Erk, pErk, p38, pp38, Jnk, pJnk, NFkB, pNFkB, and GAPDH.

resistant cancer cells. This sensitization results from increased G<sub>2</sub> arrest and apoptosis, with little P-gp inhibitory activity. Interestingly, VIC-carvedilol co-treatment can sensitize them with reduced ERK activation.

## Discussion

Drug repositioning or drug repurposing is the application of known drugs for new indications. It has been used for the treatment of various diseases and has several advantages, including low cost and minimal requirements for toxicity

testing, which is a time-consuming process (7, 8). The urgent need for pharmacological treatments for drug-resistant cancers can be efficiently addressed with drug repurposing, where these drugs can be administered to patients at a relatively faster pace. Previously, we investigated many repurposed drugs in a novel application for sensitizing drug-resistant cancer cells overexpressing P-gp to chemotherapeutic drugs. Therefore, we have also reported the potential applications of these repurposed drugs in drug-resistant cancer patients. For example, we have suggested the application of anti-malarial, anti-HIV, anti-allergic, or anti-psychotic drugs and tyrosine

kinase inhibitors for the treatment of drug-resistant cancer cells overexpressing P-gp (18-26, 32, 36, 38).

Several blood pressure-regulating drugs used for the treatment of high or low blood pressure disorders have been found to have anticancer effects and have been tested as repurposed drugs in patients with cancer (11-17). They include reserpine, amiodarone, nicardipine, carvedilol, vardenafil, quinidine and telmisartan (11-16, 39). Considering that these drugs target ion-exchange tunnels or receptors on the cell membrane and block extracellular signals, they have been suggested to target P-gp on the membranes. In particular, reserpine is a well-known P-gp inhibitor that can sensitize drug-resistant cancer cells overexpressing P-gp (9, 10).

A literature search identified 27 blood pressure regulating drugs already in use for the clinical treatment of high or low blood pressure disorders. As some of these drugs have anticancer effects, clinical trials have been conducted to test their efficacy in solid tumors (40, 41). However, comparisons of the individual blood-pressure-regulating drugs and their exact mechanisms of action have not been investigated in detail in drug-resistant cancer cells overexpressing P-gp.

Among the 27 blood-pressure-regulating drugs tested, co-treatment with four drugs (amiodarone, nicardipine, carvedilol, and vardenafil) was found to sensitize antimitotic drug-resistant KBV20C cells at relatively low doses. As ion-exchange tunnels or receptors are located in the cellular membrane and transport external signals (15, 16, 39), we assume that blood pressure-regulating drugs play a role in decreasing or modifying overexpressed P-gp activity in the membrane of drug-resistant cancer cells. Although the resistant cancer-sensitizing abilities of blood-pressure regulating drugs have been previously demonstrated (14-16), our findings represent a pioneering application of selective blood pressure-regulating drugs as repurposed drugs. Considering that patients with blood-pressure-related disorders show a much higher incidence of cancer (42), our findings might also contribute to the use of select blood pressure-regulating drugs to prevent or decrease cancer occurrence in patients with bipolar disorder. Additionally, we identified carvedilol, which can sensitize KBV20C cells treated with eribulin at relatively lower doses than amiodarone and nicardipine.

Notably, we found that amiodarone, nicardipine, carvedilol and vardenafil can sensitize KBV20C cells treated with both eribulin and VIC with similar sensitization effects. We hypothesize that they can be used in combination with other drugs targeting cancer to sensitize drug-resistant cancer cells. Moreover, eribulin was recently developed and is a promising drug for the treatment of patients in whom other anticancer drugs have failed (43-45); therefore, our results may be useful in treating eribulin-resistant cancer in the future.

A detailed analysis was performed to determine the molecular mechanisms underlying the sensitization effects of amiodarone, nicardipine, carvedilol and vardenafil. We demonstrated that co-treatment with these drugs reduced cellular proliferation and increased G<sub>2</sub> arrest in resistant KBV20C cells. Using more detailed quantitative annexin V analysis, we also demonstrated that co-treatment increased early apoptosis in the resistant KBV20C cells. Based on microscopic, FACS and annexin V analyses, we concluded that apoptosis was increased by amiodarone, nicardipine, carvedilol or vardenafil *via* increased G<sub>2</sub> arrest and reduced proliferation in drug-resistant KBV20C cells overexpressing P-gp. Furthermore, when we investigated the expression of proteins involved in cellular signaling pathways in VIC-carvedilol co-treatment, we found that ERK activation was highly reduced. This is indicative of a mechanism involving G<sub>2</sub> phase arrest *via* the ERK pathway and an increase in the number of cells undergoing apoptosis. Future detailed studies, including *in vivo* animal models, may reveal the molecular mechanisms underlying these sensitization effects. This will facilitate the quick application of amiodarone, nicardipine, carvedilol or vardenafil in patients, especially those resistant to combination therapy with antimitotic drugs. As the efflux of VIC by P-gp is the primary mechanism for the resistance of KBV20C cells, we tested whether sensitization by amiodarone, nicardipine, carvedilol or vardenafil co-treatments resulted from their P-gp inhibitory effects. We compared them with reserpine, a well-known P-gp inhibitor, as a positive control. We also demonstrated that reserpine had the highest P-gp-inhibitory activity, which was much higher than that of the well-known P-gp inhibitor verapamil. This suggests that the high sensitization effects of reserpine for co-treatment among blood pressure-regulating drugs results from the inhibitory effects of reserpine, which prevents the removal of eribulin or VIC from the cell. Interestingly, we detected little or no P-gp-inhibitory activity of amiodarone, nicardipine, carvedilol and vardenafil, suggesting that they remove or inhibit factors that block antimitotic drugs in drug-resistant cancer cells and then exert a synergistic effect in co-treated cells. Further investigations with amiodarone, nicardipine, carvedilol and vardenafil are needed to determine the molecular targets that allow sensitization without P-gp inhibition. As little or no increase in P-gp inhibition was detected for these drugs, an improved combination of chemotherapeutic agents may be developed for cancer patients who develop resistance to antimitotic drugs. As P-gp inhibitors have shown toxicity to normal cells (4, 5, 46, 47), we believe that amiodarone, nicardipine, carvedilol or vardenafil should be considered for co-treatment to sensitize drug-resistant cancer cells overexpressing P-gp. As personalized medicine is gaining popularity, our results from the study of these blood-pressure regulating drugs may contribute to making prescriptions in

drug-resistant cancer patients more effective. Such patients are generally allergic or sensitive to the P-gp-inhibitory effects in normal tissues.

## Conclusion

Our results highlight the novel selective sensitization of blood pressure-regulating drugs. Furthermore, drug-resistant KBV20C cells that overexpress P-gp can be sensitized to the antimitotic drugs eribulin or VIC by co-treatment with low doses of the repurposed drugs amiodarone, nicardipine, carvedilol or vardenafil. Notably, reserpine (a positive control), which has a very high P-gp inhibitory activity, provides the best sensitization of drug-resistant cancer cells when compared to the other 27 blood-pressure-regulating drugs studied. As the toxicities of these drugs are already documented, they are readily available for clinical use. Our results could contribute to the improved efficacy of various chemotherapeutic agents, when used in combination with these sensitizing drugs, for the treatment of cancer patients who develop resistance to chemotherapeutic drugs *via* P-gp overexpression.

## Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

## Authors' Contributions

Chunxue Jiang and Tian Zheng: Collected data, contributed data or analysis tools, wrote the article. Jae Hyeon Park, Jin-Sol Lee, Yunmoon Oh and Amit Kundu: Contributed data or analysis tools. Hyung Sik Kim and Sungpil Yoon: Contributed data or analysis tools, conceived and designed the analysis, collected data, contributed data or analysis tools, wrote the article.

## Acknowledgements

We thank Ji Young Kim for their technical support and preparation of the manuscript. This work was supported by National Research Foundation of Korea (NRF) funded by the Ministry of Education (NRF-2019R1A2C2002923).

## References

- Jordan MA and Wilson L: Microtubules as a target for anticancer drugs. *Nat Rev Cancer* 4(4): 253-265, 2004. PMID: 15057285. DOI: 10.1038/nrc1317
- McGrogan BT, Gilmartin B, Carney DN and McCann A: Taxanes, microtubules and chemoresistant breast cancer. *Biochim Biophys Acta* 1785(2): 96-132, 2008. PMID: 18068131. DOI: 10.1016/j.bbcan.2007.10.004
- Kim JH, Yoo HI, Kang HS, Ro J and Yoon S: Salinomycin sensitizes antimitotic drugs-treated cancer cells by increasing apoptosis *via* the prevention of G2 arrest. *Biochem Biophys Res Commun* 418(1): 98-103, 2012. PMID: 22244892. DOI: 10.1016/j.bbrc.2011.12.141
- Chen Z, Shi T, Zhang L, Zhu P, Deng M, Huang C, Hu T, Jiang L and Li J: Mammalian drug efflux transporters of the ATP binding cassette (ABC) family in multidrug resistance: A review of the past decade. *Cancer Lett* 370(1): 153-164, 2016. PMID: 26499806. DOI: 10.1016/j.canlet.2015.10.010
- Shukla S, Wu CP and Ambudkar SV: Development of inhibitors of ATP-binding cassette drug transporters: present status and challenges. *Expert Opin Drug Metab Toxicol* 4(2): 205-223, 2008. PMID: 18248313. DOI: 10.1517/17425255.4.2.205
- Yoon S, Wang X, Vongpunasawad S, Tromp G and Kuivaniemi H: Editorial: FDA-approved drug repositioning for P-glycoprotein overexpressing resistant cancer. *Front Oncol* 11: 632657, 2021. PMID: 33816271. DOI: 10.3389/fonc.2021.632657
- Clark KB: New therapeutic bearings for repositioned drugs. *Curr Top Med Chem* 13(18): 2281-2282, 2013. PMID: 24059466. DOI: 10.2174/15680266113136660159
- Pantziarka P and Cairns L: Recycling existing drugs for cancer therapy: delivering low cost cancer care. *Ecancermedicalscience* 8: ed40, 2014. PMID: 25075221. DOI: 10.3332/ecancer.2014.ed40
- Bhat UG, Winter MA, Pearce HL and Beck WT: A structure-function relationship among reserpine and yohimbine analogues in their ability to increase expression of *mdr1* and P-glycoprotein in a human colon carcinoma cell line. *Mol Pharmacol* 48(4): 682-689, 1995. PMID: 7476894.
- Sarver JG, Klis WA, Byers JP and Erhardt PW: Microplate screening of the differential effects of test agents on Hoechst 33342, rhodamine 123, and rhodamine 6G accumulation in breast cancer cells that overexpress P-glycoprotein. *J Biomol Screen* 7(1): 29-34, 2002. PMID: 11897053. DOI: 10.1177/108705710200700105
- Takara K, Sakaeda T and Okumura K: Carvedilol: a new candidate for reversal of MDR1/P-glycoprotein-mediated multidrug resistance. *Anticancer Drugs* 15(4): 303-309, 2004. PMID: 15057133. DOI: 10.1097/00001813-200404000-00001
- Tatosian DA and Shuler ML: A novel system for evaluation of drug mixtures for potential efficacy in treating multidrug resistant cancers. *Biotechnol Bioeng* 103(1): 187-198, 2009. PMID: 19137589. DOI: 10.1002/bit.22219
- Lee SY, Rhee YH, Jeong SJ, Lee HJ, Lee HJ, Jung MH, Kim SH, Lee EO, Ahn KS, Ahn KS and Kim SH: Hydrocinchonine, cinchonine, and quinidine potentiate paclitaxel-induced cytotoxicity and apoptosis *via* multidrug resistance reversal in MES-SA/DX5 uterine sarcoma cells. *Environ Toxicol* 26(4): 424-431, 2011. PMID: 20196146. DOI: 10.1002/tox.20568
- Figueroa-González G, Jacobo-Herrera N, Zentella-Dehesa A and Pereda-Miranda R: Reversal of multidrug resistance by morning glory resin glycosides in human breast cancer cells. *J Nat Prod* 75(1): 93-97, 2012. PMID: 22148475. DOI: 10.1021/np200864m
- Englund G, Lundquist P, Skogastierna C, Johansson J, Hoogstraate J, Afzelius L, Andersson TB and Projean D: Cytochrome p450 inhibitory properties of common efflux transporter inhibitors. *Drug Metab Dispos* 42(3): 441-447, 2014. PMID: 24396142. DOI: 10.1124/dmd.113.054932
- Kathawala RJ, Wang YJ, Ashby CR Jr and Chen ZS: Recent advances regarding the role of ABC subfamily C member 10 (ABCC10) in the efflux of antitumor drugs. *Chin J Cancer* 33(5): 223-230, 2014. PMID: 24103790. DOI: 10.5732/cjc.013.10122

- 17 López Brunsó M, Toro Blanch C, Sais Girona E, Roa García D, Hernández Martínez A, Izquierdo Font A, Guerra Prió S, Mas Pueyo HG and Bosch-Barrera J: Probable drug-drug interaction between erlotinib and amiodarone causes severe neurotoxicity in a patient with advanced lung cancer. *Anticancer Drugs* 29(4): 380-383, 2018. PMID: 29420339. DOI: 10.1097/CAD.0000000000000600
- 18 Jiang C, Lee SH, Park JH, Lee JS, Park JW, Kim JR, Lee SH, Kim HS and Yoon S: A low dose of aripiprazole has the strongest sensitization effect among 19 repositioned bipolar drugs in P-gp-overexpressing drug-resistant cancer cells. *Anticancer Res* 41(2): 687-697, 2021. PMID: 33517273. DOI: 10.21873/anticancer.14820
- 19 Kim KS, Jiang C, Kim JY, Park JH, Kim HR, Lee SH, Kim HS and Yoon S: Low-dose crizotinib, a tyrosine kinase inhibitor, highly and specifically sensitizes P-glycoprotein-overexpressing chemoresistant cancer cells through induction of late apoptosis *in vivo* and *in vitro*. *Front Oncol* 10: 696, 2020. PMID: 32528877. DOI: 10.3389/fonc.2020.00696
- 20 Park Y, Son JY, Lee BM, Kim HS and Yoon S: Highly eribulin-resistant KBV20C oral cancer cells can be sensitized by co-treatment with the third-generation P-glycoprotein inhibitor, elacridar, at a low dose. *Anticancer Res* 37(8): 4139-4146, 2017. PMID: 28739698. DOI: 10.21873/anticancer.11801
- 21 Kim JY, Kim HS and Yoon S: Tyrosine kinase inhibitors imatinib and erlotinib increase apoptosis of antimitotic drug-resistant KBV20C cells without inhibiting P-gp. *Anticancer Res* 39(7): 3785-3793, 2019. PMID: 31262905. DOI: 10.21873/anticancer.13527
- 22 Kim JY, Kim KS, Kim IS and Yoon S: Histamine receptor antagonists, loratadine and azelastine, sensitize P-gp-overexpressing antimitotic drug-resistant KBV20C cells through different molecular mechanisms. *Anticancer Res* 39(7): 3767-3775, 2019. PMID: 31262903. DOI: 10.21873/anticancer.13525
- 23 Kim JY, Park YJ, Lee BM and Yoon S: Co-treatment with HIV protease inhibitor nelfinavir greatly increases late-phase apoptosis of drug-resistant KBV20C cancer cells independently of P-glycoprotein inhibition. *Anticancer Res* 39(7): 3757-3765, 2019. PMID: 31262902. DOI: 10.21873/anticancer.13524
- 24 Kim JY, Park Y, Lee BM, Kim HS and Yoon S: P-gp inhibition by the anti-psychotic drug pimozide increases apoptosis, as well as expression of pRb and pH2AX in highly drug-resistant KBV20C cells. *Anticancer Res* 38(10): 5685-5692, 2018. PMID: 30275188. DOI: 10.21873/anticancer.12905
- 25 Kim JY, Son JY, Lee BM, Kim HS and Yoon S: Aging-related repositioned drugs, donepezil and sildenafil citrate, increase apoptosis of anti-mitotic drug-resistant KBV20C cells through different molecular mechanisms. *Anticancer Res* 38(9): 5149-5157, 2018. PMID: 30194162. DOI: 10.21873/anticancer.12837
- 26 Kim JY, Tae IH, Lee BM, Kim HS and Yoon S: Low doses of the anti-psychotic drug aripiprazole have strong P-gp-inhibitory activity and sensitize anti-mitotic drug-resistant cancer cells. *Anticancer Res* 38(9): 5101-5108, 2018. PMID: 30194155. DOI: 10.21873/anticancer.12830
- 27 Cheon JH, Kim JY, Lee BM, Kim HS and Yoon S: P-gp inhibition by XL019, a JAK2 inhibitor, increases apoptosis of vincristine-treated resistant KBV20C cells with increased p21 and pH2AX expression. *Anticancer Res* 37(12): 6761-6769, 2017. PMID: 29187454. DOI: 10.21873/anticancer.12136
- 28 Cheon JH, Kim KS, Yadav DK, Kim M, Kim HS and Yoon S: The JAK2 inhibitors CEP-33779 and NVP-BSK805 have high P-gp inhibitory activity and sensitize drug-resistant cancer cells to vincristine. *Biochem Biophys Res Commun* 490(4): 1176-1182, 2017. PMID: 28669723. DOI: 10.1016/j.bbrc.2017.06.178
- 29 Choi AR, Kim JH, Woo YH, Cheon JH, Kim HS and Yoon S: Co-treatment of LY294002 or MK-2206 with AZD5363 attenuates AZD5363-induced increase in the level of phosphorylated AKT. *Anticancer Res* 36(11): 5849-5858, 2016. PMID: 27793908. DOI: 10.21873/anticancer.11170
- 30 Kim M, Jee SC, Kim KS, Kim HS, Yu KN and Sung JS: Quercetin and isorhamnetin attenuate benzo[a]pyrene-induced toxicity by modulating detoxification enzymes through the AhR and NRF2 signaling pathways. *Antioxidants (Basel)* 10(5): 787, 2021. PMID: 34065697. DOI: 10.3390/antiox10050787
- 31 Kim KS, Lee JS, Park JH, Lee EY, Moon JS, Lee SK, Lee JS, Kim JH and Kim HS: Identification of novel biomarker for early detection of diabetic nephropathy. *Biomedicines* 9(5): 457, 2021. PMID: 33922243. DOI: 10.3390/biomedicines9050457
- 32 Choi AR, Kim JH, Woo YH, Kim HS and Yoon S: Anti-malarial drugs primaquine and chloroquine have different sensitization effects with anti-mitotic drugs in resistant cancer cells. *Anticancer Res* 36(4): 1641-1648, 2016. PMID: 27069141.
- 33 Cheon JH, Lee BM, Kim HS and Yoon S: Highly Halaven-resistant KBV20C cancer cells can be sensitized by co-treatment with fluphenazine. *Anticancer Res* 36(11): 5867-5874, 2016. PMID: 27793910. DOI: 10.21873/anticancer.11172
- 34 Choi AR, Kim JH, Cheon JH, Kim HS and Yoon S: Attenuation of colchicine toxicity in drug-resistant cancer cells by co-treatment with anti-malarial drugs. *Anticancer Res* 36(11): 5859-5866, 2016. PMID: 27793909. DOI: 10.21873/anticancer.11171
- 35 Florian S and Mitchison TJ: Anti-microtubule drugs. *Methods Mol Biol* 1413: 403-421, 2016. PMID: 27193863. DOI: 10.1007/978-1-4939-3542-0\_25
- 36 Lim JS, Park Y, Lee BM, Kim HS and Yoon S: Co-treatment with celecoxib or NS398 strongly sensitizes resistant cancer cells to antimitotic drugs independent of P-gp inhibition. *Anticancer Res* 36(10): 5063-5070, 2016. PMID: 27798865. DOI: 10.21873/anticancer.11075
- 37 Wang LG, Liu XM, Kreis W and Budman DR: The effect of antimicrotubule agents on signal transduction pathways of apoptosis: a review. *Cancer Chemother Pharmacol* 44(5): 355-361, 1999. PMID: 10501907. DOI: 10.1007/s002800050989
- 38 Choi AR, Kim JH and Yoon S: Thioridazine specifically sensitizes drug-resistant cancer cells through highly increase in apoptosis and P-gp inhibition. *Tumour Biol* 35(10): 9831-9838, 2014. PMID: 24989930. DOI: 10.1007/s13277-014-2278-1
- 39 Weiss J, Sauer A, Divac N, Herzog M, Schwedhelm E, Böger RH, Haefeli WE and Benndorf RA: Interaction of angiotensin receptor type 1 blockers with ATP-binding cassette transporters. *Biopharm Drug Dispos* 31(2-3): 150-161, 2010. PMID: 20222053. DOI: 10.1002/bdd.699
- 40 Bates SE, Meadows B, Goldspiel BR, Denicoff A, Le TB, Tucker E, Steinberg SM and Elwood LJ: A pilot study of amiodarone with infusional doxorubicin or vinblastine in refractory breast cancer. *Cancer Chemother Pharmacol* 35(6): 457-463, 1995. PMID: 7882454. DOI: 10.1007/BF00686829
- 41 Chen N, Weiss D, Reyes J, Liu L, Kasserra C, Wang X, Zhou S, Kumar G, Weiss L and Palmisano M: No clinically significant drug interactions between lenalidomide and P glycoprotein substrates and inhibitors: results from controlled phase I studies in healthy volunteers. *Cancer Chemother Pharmacol* 73(5): 1171-1179, 2014. PMID: 24588888. DOI: 10.1007/s00280-014-2588-1

- 1031-1039, 2014. PMID: 24659021. DOI: 10.1007/s00280-014-2438-4
- 42 Padala SA, Barsouk A, Thandra KC, Saginala K, Mohammed A, Vakiti A, Rawla P and Barsouk A: Epidemiology of renal cell carcinoma. *World J Oncol* 11(3): 79-87, 2020. PMID: 32494314. DOI: 10.14740/wjon1279
- 43 Dell'Ova M, De Maio E, Guiu S, Roca L, Dalenc F, Durigova A, Pinguet F, Bekhtari K, Jacot W and Pouderoux S: Tumour biology, metastatic sites and taxanes sensitivity as determinants of eribulin mesylate efficacy in breast cancer: results from the ERIBEX retrospective, international, multicenter study. *BMC Cancer* 15: 659, 2015. PMID: 26449988. DOI: 10.1186/s12885-015-1673-3
- 44 Dybdal-Hargreaves NF, Risinger AL and Mooberry SL: Eribulin mesylate: mechanism of action of a unique microtubule-targeting agent. *Clin Cancer Res* 21(11): 2445-2452, 2015. PMID: 25838395. DOI: 10.1158/1078-0432.CCR-14-3252
- 45 Inoue K, Saito T, Okubo K, Kimizuka K, Yamada H, Sakurai T, Ishizuna K, Hata S, Kai T and Kurosumi M: Phase II clinical study of eribulin monotherapy in Japanese patients with metastatic breast cancer who had well-defined taxane resistance. *Breast Cancer Res Treat* 157(2): 295-305, 2016. PMID: 27125669. DOI: 10.1007/s10549-016-3808-x
- 46 Chufan EE, Kapoor K and Ambudkar SV: Drug-protein hydrogen bonds govern the inhibition of the ATP hydrolysis of the multidrug transporter P-glycoprotein. *Biochem Pharmacol* 101: 40-53, 2016. PMID: 26686578. DOI: 10.1016/j.bcp.2015.12.007
- 47 Yang K, Wu J and Li X: Recent advances in the research of P-glycoprotein inhibitors. *Biosci Trends* 2(4): 137-146, 2008. PMID: 20103919.

*Received October 5, 2021*

*Revised November 11, 2021*

*Accepted November 15, 2021*