Original Article

Reduce Doxorubicin Resistant by Inhibiting ABC Efflux Transporters Proteins

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Abstract

Background: Many aspects of medication absorption are impacted by ATP-binding cassette efflux transporters (ABC). These efflux protein restrict the availability of many different chemicals, including those used to treat cancer. Doxorubicin is one of the common anticancer drugs that suffering from resistant and low cell penetration. **Methods**: In this study, assessing the permeability of two BCRP substrates, pantoprazole and doxorubicin, in the rat everted gut, the effects of resveratrol and vinblastine on BCRP efflux activity were determined. In addition, Caco-2 cells were utilized to investigate the impact of BCRP inhibition on doxorubicin uptake by cells. The concentrations of pantoprazole and doxorubicin that absorbed through the intestinal membrane of everted gut were measured by HPLC with or without resveratrol and vinblastine at several time interval (10, 20, 30, 40 and 60 min) by using in vitro everted gut. **Results:** Incubation for 60 min showed the penetration of pantoprazole with resveratrol and vinblastine was significantly increased by 1.6 and 1.5- fold. Furthermore, doxorubicin that penetrates over the intestine membrane raised significantly ($p \leq 0.001$) while it was treated with pantoprazole, by 1.8. Where are resveratrol and vinblastine increase doxorubicin absorbency by 1.6 and 1.5 fold, respectively. Caco-2 cells uptake of doxorubicin was raised when pantoprazole was present, resveratrol and vinblastine by 1.9 fold, 1.65 fold and 1.5 fold, correspondingly. **Conclusion:** This current study is a novel research which showed the inhibitory impact of resveratrol and vinblastine on BCRP-protein efflux activity in GIT tract and thus increases the doxorubicin bioavailability.

Keywords: BCRP, Doxorubicin, Multidrug Resistance, Everted Gut, Resveratrol and Vinblastine.

Introduction

Different families of ATP-binding cassette transporters (ABC) efficiently move a varied range of substrates within many membranes in human and animals, including, peptides, nutrients , metabolic components , toxic substances, and pharmaceuticals drugs.[1] Intestinal epithelial cells express intensively breast cancer resistant protein (ABCG2), multidrug resistance protein (ABCC1), and p-glycoprotein (ABCB1) as efflux transporters proteins.[2,3]

The process of multidrug resistance especially for anticancer drugs is significantly influenced by certain ABC transporter members.

Increase expression of ABC drug efflux pump proteins in many cancer cells for instance p-gp and BCRP and MRP is a crucial technique induced by cancer cells to survive by removing the anticancer medication outside the cancer cells, thus rendering anticancer medications ineffective and, induce MDR.[4] This is a complex issue by which cancer cells develop cross resistant to numerous drugs diverse structurally and pharmacologically such as vinblastine, paclitaxel, and doxorubicin.[5]

BCRP is expressed generally in small intestine cells membranes, brain, breast, prostate, and ovaries.[6] Chemotherapy drugs absorption is significantly reduced due to the expression of BCRP in intestine which leads to reduce drugs bioavailability.[7]

Doxorubicin, an extensively used drug for numerous kinds of cancers, for instance breast cancer, GI cancer, leukaemia, bone cancer.[8] Doxorubicin is a substrate of some members of ABC efflux proteins like BCRP. Though, the efficacy of doxorubicin is often conceded due to poor absorption, low bioavailability and the progress of drug

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resistance by cancer cells that present major struggle to get a successful cancer control.[9,10] For that, more researches need to focus to increase anticancer drugs bioavailability by combining them with ABC inhibitors.

Many natural substances are recognized for their medical use like anticancer activity and at the same time, their capability to inhibit ABC efflux activity.[11] Reduction of BCRP function by medical herbs is representing as an innovative way for encountering and reducing MDR without producing undesirable toxic effects.

Resveratrol is a polyphenol isolated from natural plant (veratrum grandiflorum) and is commonly found in many plants like grape, berry, and peanut.[12] Resveratrol has numerous biological roles, like reduce oxidative stress (antioxidant), diminish inflammation (anti-inflammation), protective effect against cancer (anti-tumor), increase immunity and anti- lower coronary heart disease.[13,14] Resveratrol has a great efficacy for cancer treatment that approved in scientific studies that carried out by using *in vitro* with *in vivo* models.[15,16] In addition to its ability to reduce inflammation and free radical, make it an excellent choice to be administrated to cancer paints. Moreover, resveratrol could inhibit many ABC efflux pump like p-gp and BCRP that could help to reduce MDR.[17] As a result, all these characteristics made it a good choice to examine in drug resistant.

Recently, researches have approved that vinblastine (natural Vinca alkaloid) is useful herbal drug used for different type of cancer treatment. It adheres to tubulin in the nucleus and stops microtubule formation, initiating disturbing to the mitotic spindle and stop tumour cells to be divided.[18] Vinblastine is known as a substrate to ABC efflux transporter and it showed a great affinity to bind with p-gp and MRP2 in GIT track.[19]

In the current study, the important effects of pantoprazole (BCRP inhibitors), resveratrol and vinblastine on the function of BCRP to efflux anticancer drugs like doxorubicin out of the intestine cell are examined by using commonly identified *in vitro* model of everted gut and Caco-2 cells.

Materials and Methodology

Materials

All drugs, other chemicals and solvent were obtained from Sigma Aldrich, US. Resveratrol and vinblastine on were obtained from Thermo Scientific (U.S.A). Caco-2 cells were purchase from the ATCC (ATCC, USA).

All chemical reagents utilised in this experimental studies were bough suitable of analytical grade.

Rat Everted Sacs Preparation

Using everted sac technique was applied in accordance with published research.[20] The University of Baghdad's local ethics council approved all rat studies (Baghdad, Iraq). Sprague-Dawley rats with weight range between 190 and 250 g were allowed full access to water and fasted for at least 24 hours. The intestines' jejunum was removed from the rat while it was under anaesthesia; the section was then repeatedly cleaned with regular 0.9 % NaCl saline solution which preserved in ringer buffer supplied with oxygen. To prepare ringer solution, 10 mM glucose, 155 NaCl, 4 KCl, 2 $CaCl_2$ and 1.2 $MgCl_2$, were added and dissolved in distilled water. The intestines were filled with oxygenated buffer and everted on a glass rod. Each was then separated into sacs that were 3.4–4.2 cm long by utilizing silk sutures. Throughout the experiment, the sacs were maintained in a buffer (oxygenated) and incubated with 5% CO_2 at 37 °C.

BCRP-transporter Study

In 20 millilitres of oxygenated ringer solutions, the sacs were placed in the following combinations: vinblastine (50 µM), pantoprazole (100 µM) (control), doxorubicin (2 µM) alone, pantoprazole and doxorubicin together, or pantoprazole or doxorubicin plus resveratrol (30 µM). Vinblastine and resveratrol concentrations has been chosen according to preliminary study.

Sacs were taken out at different intervals (10, 20, 30, 40, and 60 minutes), repeatedly cleaned in regular saline, and the serosal contents were gathered. Before being analysed, samples are kept in the refrigerator.

A validated HPLC method was used to measure the amounts of doxorubicin and pantoprazole. To analyse the volume of the fluid inside each sac, the weight of each sac was noted both before and after the experiment.

Evaluation of Everted Gut Sacs Viability

Using a monitor (One Touch, UK), glucose level at inside and outside the gastrointestinal sac were examined after 60 min so that assess the condition and feasibility of the intestinal gut sac.

Remove out sacs from the water bath. Snip one of the sac end and collect the contents into a fresh plastic tube. In addition, gather a 1-ml sample of the incubation liquid and transfer it to another plastic test tube. Dilute the sample with the same volume of water beforehand measuring glucose concentration. Assess the glucose concentration of both inside and outside the sacs solutions using a glucometer.

The formation of lactic dehydrogenase enzyme (LDH), which was tested by a scientific laboratory (Iraq) using an LDH kit, was used to assess the cell damage. The LDH test relies on the transformation of lactate into pyruvate facilitated by LDH, concurrently reducing NAD to NADH. The alteration in absorbance was measured (at 440 nm) by a microplate spectrophotometer setup. U/L/cm^2 of sac area was used to calculate the results.

Cell Culture

Plastic culture flasks were used to preserve Caco-2 cells that were obtained from the ATCC (American Type Culture Collection USA) and used in assays at passage 15–25. The stock cells were sub-cultured usually before they reaching -full confluence.

Upon reaching confluence, the Caco-2 cells were collected using 0.25 mM trypsin and 0.2% EDTA (for 1 to 2 min

at 37°C degree), then cells resuspended and transferred to a new flask.

The medium was Dulbecco's Modified Eagle's Medium with foetel bovine serum (20%), glutamax (1%), non-essential amino acid (1%), and sodium pyruvate (1%) added as supplements. The monolayer cultures were cultivated with 5% CO2 at 37°C in a humidified condition and the media was replaced every 48 h. Routine cell function and activity tests were carried out to insure that the Coca-2 cells still preserve the important enzymes and function normally. The enzymes tests examined as follow:

Glutathione Peroxidase (GPx) Enzymatic Assay

Coca-2 Cell physiological function was carried out by testing the activity of cellular glutathione peroxidase which was assessed using the GPx cellular activity assay kit. This kit employs an indirect approach, centered on the conversion of reduced glutathione to oxidized glutathione facilitated by GPx. Oxidation of NADPH to NADP is indicative of this enzyme activity. The level of NADPH was measured at 412 nm and GSH-Px activity was reported in international units per mg of soluble cellular proteins.

Superoxide Dismutase (SOD) Assay

The activity of cellular superoxide dismutase (SOD) was assessed in Caco-2 cells utilizing the SOD activity assay kit, which is founded on compete between the oxidation of pyrogallol driven by superoxide radicals and the dismutation of superoxide facilitated by SOD. Absorbance readings were taken at 550 nm with a microplate spectrophotometer. A unit of SOD activity is characterized as the quantity of enzyme necessary to reduce the rate of auto-oxidation of pyrogallol by half.

Alkaline Phosphatase

The enzymatic activity of alkaline phosphatase is a marker enzyme for Coca-2 cells permeability integrity.[21] It was assessed using an alkaline phosphatase assay kit. This test relies on observing the shift in color as paranitrophenol phosphate, which is initially colorless, transforms into paranitrophenol, a yellow compound. The activity was quantified with a microplate reader set to 520 nm.

MTT Assay for Cell Viability Test

To examine cell viability, MTT assay was used as described in previous studies.[22]

A 100 µl of Caco-2 cells were placed in 96-well plates with a density of $1x10^4$ cells per each well with culture medium consist of DMEM enriched with fetal bovine serum (10%) and Penicillin/Streptomycin (1%), excluding the final row which solely contained 100 µl of DMEM and served as the control. The cells were then incubated at 37 °C with 95% humidity and 5% CO2 for 24 h. At the day of the experiment, pantoprazole, doxorubicin, resveratrol, and vinblastine were introduced into each well at concentrations of 100 µM, 2 µM, 30 µM, and 50 µM, respectively. Additionally, dimethyl sulfoxide (DMSO) and incubation buffer were utilized as positive control and negative control, respectively. Subsequent a 24-h incubation time, media war replaced with 50 µL of MTT solution (4 mg/mL) was added to every well, including the controls. Afterwards 3 h of incubation (at 37°C), the supernatant was discarded, and 100 µL of DMSO was added with agitation for 6 min at normal room temperature. Ultimately, the absorbance at 570 nm was recorded using a microtiter plate reader. Cell viability is represented as a percentage of each treatment to the control group.

Drug Uptake Study in Caco-2 Cell

Caco-2 cells were cultured on 12-well plastic plates at a cell density of 1×10^5 cells/ cm^2 for the uptake investigations. The cell monolayers were utilised for the uptake tests at 5–7 days after being supplied a new growth medium every two to three days.

The Caco-2 cells were exposed to pantoprazole, resveratrol and vinblastine at concentrations of 100 µM, 30 µM and 50 µM, respectively for 60 min. A preliminary study has been carried out to figure out the more effective concentrations of resveratrol and vinblastine to inhibit BCRP activity with no toxic impact, and according to that, the above concentration have been chosen.

The uptake investigation was conducted using HBSS-MES (pH 5.0) buffer as the incubation medium. 1.0 mL of incubation medium containing a 2 µM of doxorubicin substrate was added after the medium was removed. At the conclusion of the incubation period, each cell monolayer was quickly rinsed twice using 2.0 mL of ice-cold incubation solution. 0.6 mL of 1 N NaOH was used to dissolve the cells, and 0.5 mL of HCl was used to neutralise them.

Following a thorough vortex, 150 mL of the mixture was moved to a fresh tube, then 500 mL of MeOH was added. The medicines concentration in the supernatant was measured by HPLC following a 15-minute, 14,000×g centrifugation of the combination.

HPLC Samples Analysis

Pantoprazole and doxorubicin concentrations were determined by a HPLC instrument.

The HPLC apparatus (Thermo Separation Products, USA) was used to analyse pantoprazole samples at a wavelength of 200 nm using a C18 column (4.6 x 150 mm, 5 m, Milford, USA) at a flow rate of 1 mL/min. 45% acetonitrile and 0.03 mol/L KH2PO4 with a pH of 2.5 were included in the mobile phase.[23]

For doxorubicin sample, the HPLC was fixed at 482/550 nm wavelength -Fluorescent Detector and coupled to the on a 50 ×1 mm I.D. (5 m) reversed phase C18 column, Luna type (Phenomenex ,USA). Components of the mobile phase include acetonitrile and heptanesulfonic acid (0.2%, pH 4) in a 25:75 ratio, employing a gradient elution technique. Specifically, the flow rate was set at 1 mL/min for the first 13 minutes, gradually increasing to 1.5 mL/min until the 25-minute mark, after which it

was sustained for 9 minutes before reverting to 1 mL/min over a one-minute period.[24]

All samples of pantoprazole and doxorubicin concentrations that measured in this study were higher than the lower limit of detection.

Statistical Analysis

Each test was conducted five times. Values are expressed as mean ± SD (standard deviation). Statistical analyses were determined by software SPSS v22 to execute unpaired student t-tests. To consider as a significant change a P-value must be less or equal to 0.05 (≤ 0.05).

Results

Everted Gut Evaluation

The functional activity of everted sacs was assessed by analyzing glucose levels on two sides, specifically across mucosal (inside the sac) and serosal (outside the sac) fluid. Throughout the trials, at 60 minutes, the glucose level in the serosal fluid was around 1.7 times greater than that in the mucosal fluid. This result clearly indicates that glucose absorption is an active process, confirming that the everted gut sacs remained well intact and functional throughout the entire experimental time. Furthermore, LDH functional activity was evaluated as an extra indicator of sac feasibility. The average LDH functional activity observed in the incubation solution was roughly 150 ± 10 U/L/cm². At 10, 20, 30, 40, and 60 minutes, no significant discrepancies were noted among the different time points, demonstrating that the viability of the sacs was consistently maintained throughout the experiment.

Influence of Drugs on BCRP Functional Activity Effect of Resveratrol and Vinblastine on Pantoprazole Absorption

To approve that the BCRP transporter protein is active and functional in the everted gut through the experiments duration, studies were conducted to examine the effect of resveratrol and vinblastine presence on pantoprazole (used as a known BCRP substrate and inhibitor)[25] transport by BCRP within the intestine wall.

The penetration of pantoprazole through the intestine membrane in the presence of doxorubicin, resveratrol and vinblastine enhanced in time dependent manner. An initial study has been done to determine the best effective and safe concentrations of resveratrol and vinblastine to inhibit BCRP activity (data not showen).

At all-time point started from 5 to 60 min treatment of intestinal sacs with pantoprazole, the results displayed a low concentration of pantoprazole could penetrate through the intestinal epithelium. Figure 1 demonstrate, incubation of everted sac with doxorubicin, resveratrol and vinblastine at concentration of 30 µM and 50 µM, respectively significantly rise pantoprazole absorbency by 1.8- fold, 1.7-fold and 1.5–fold, separately in comparison to pantoprazole ($P < 0.01$).

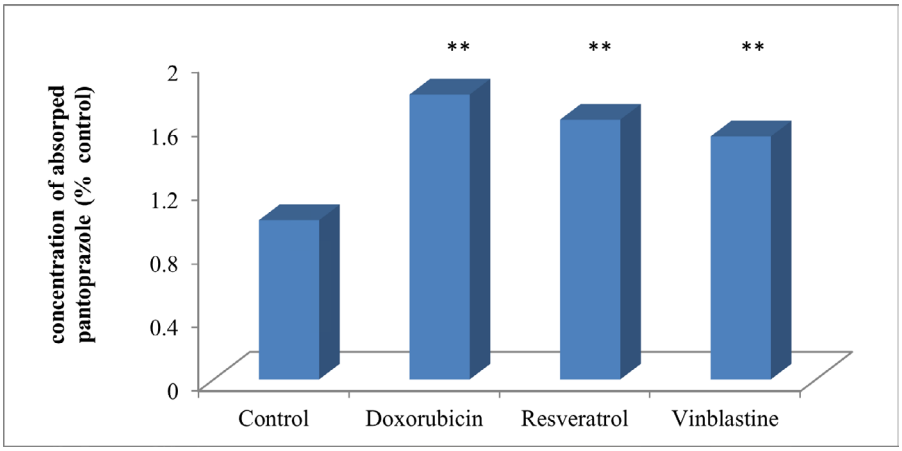

Figure 1: The Effect of BCRP Inhibitors (doxorubicin, resveratrol and vinblastine) on Pantoprazole Permeability at 60 Min Incubation In Everted Rat Intestinal Sac.

Impact of Resveratrol and Vinblastine on Doxorubicin Absorption

Additional investigations were performed to determine how pantoprazole, resveratrol and vinblastine influenced the uptake of doxorubicin through the walls of everted intestinal segments. When intestinal sacs were treated with doxorubicin alone, the uptake rate was found to be low because of the efflux mechanisms of ABC transporters. The penetration of doxorubicin within the gut sacs significantly elevated with the incorporation of pantoprazole, resveratrol, and vinblastine, with this enhancement linked to the extended incubation time. After a 60-minute exposure of the gut sacs with the BCRP inhibitor pantoprazole (100 µM), the permeability of doxorubicin exhibited a notable rise, increasing by 1.8 times ($P < 0.01$) in comparison with doxorubicin alone (control). Conversely, treatment with resveratrol (30 µM) and vinblastine (50 µM) resulted in substantial enhancements in doxorubicin penetration ($P < 0.01$) by 1.6 and 1.5 times, respectively, when compared to doxorubicin alone (Figure2).

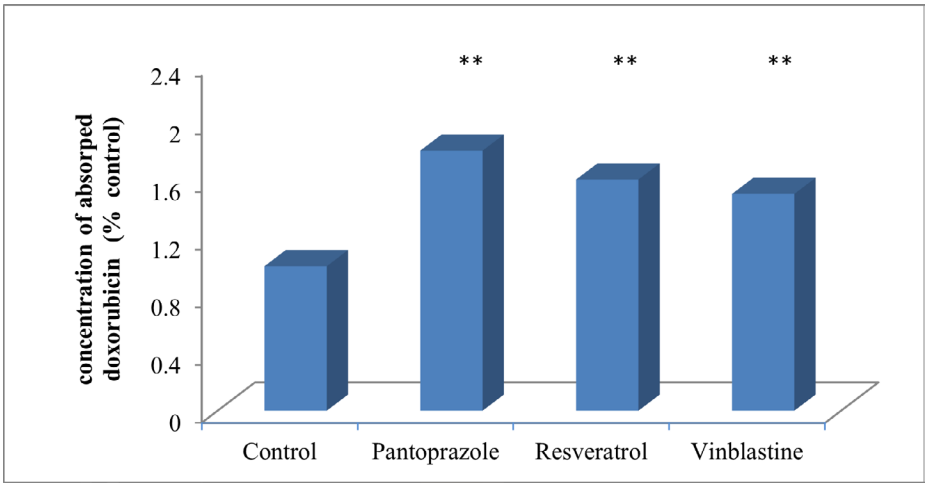

Figure 2: The Effect of BCRP Inhibitors (pantoprazole, resveratrol and vinblastine) on Doxorubicin Permeability at 60 Min Incubation in Everted Rat Intestinal Sac.
Note: P<0.01

Coca-2 Cells Characterization

Initial experiments were carried out routinely to ensure the Coca-2 cell still preserve the enzymes active and the physiological functions after many passages, Glutathione Peroxidase assay, Superoxide Dismutase (SOD) assay and Alkaline Phosphatase assay were determined.

The results of the above test showed that there are no significant differences between the fresh bought Coca-2 cells and between Coca-2 passages that used in this study. The results are presented in table 1.

Table 1: Activities of Glutathione Peroxidase, Superoxide Dismutase and Alkaline Phosphatase in New Coca-2 and Passage 15-25 Coca-2 Cells.

Enzymes	New Coca-2 Cells	Coca-2 Passage 15–25
Glutathione Peroxidase (U mg−1 cell protein)	203.7 ± 1.01	198 ± 1.21
Superoxide Dismutase (U mg−1 cell protein)	27.2 ± 0.98	26.99 ± 0.87
Alkaline Phosphatase	102 ± 0.78	101 ± 0.85

Impact of Drugs on the Availability of Caco-2 Cells

The viability of Caco-2 incubated to pantoprazole, doxorubicin, resveratrol and vinblastine was measured using the MTT assay. There was no significant toxicity observed in cells that were incubated 100 µM pantoprazole, 2 µM doxorubicin, 30 µM resveratrol and 50 µM vinblastine after a 24 h incubation, figure 3.

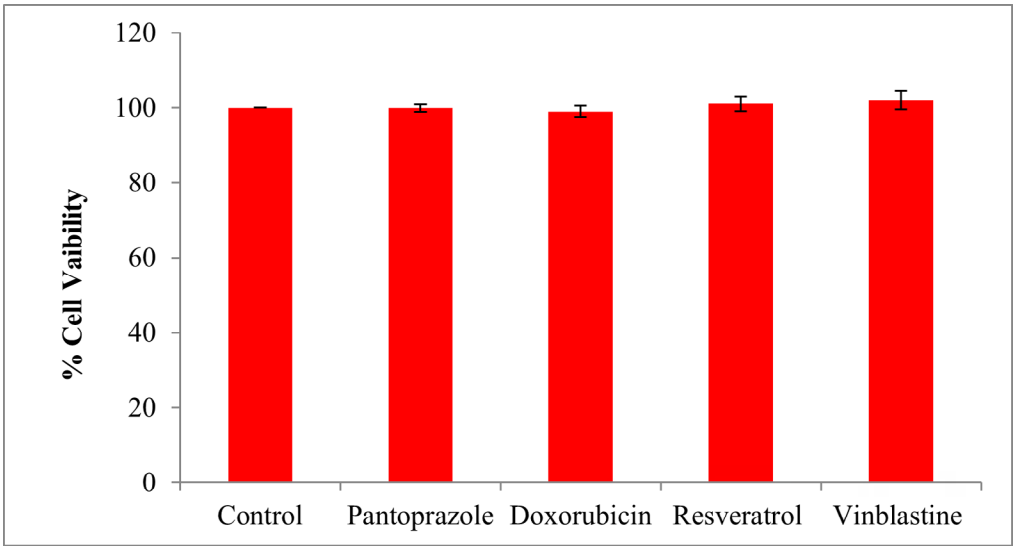

Figure 3: The effect of Pantoprazole, Doxorubicin, Resveratrol and Vinblastine on Coca-2 Cell Viability.

Impact of BCRP Inhibitors on the Efflux of Doxorubicin Through Caco-2 Cells

An uptake experiments were carried out of Coca-2 cells to describe the efflux transport of doxorubicin. The amount of doxorubicin that remained in Caco-2 cells after 60 minutes in cells incubated with inhibitors (pantoprazole, resveratrol and vinblastine) was used to characterise the efflux of doxorubicin. The uptake of doxorubicin reached an almost stable state 30 minutes after the start of incubation. When pantoprazole, resveratrol and vinblastine were present, the level of doxorubicin inside the Coca-2 cell was significantly increase by 1.9 fold, 1.65 fold and 1.5 fold, respectively in comparison to doxorubicin alone, figure 4.

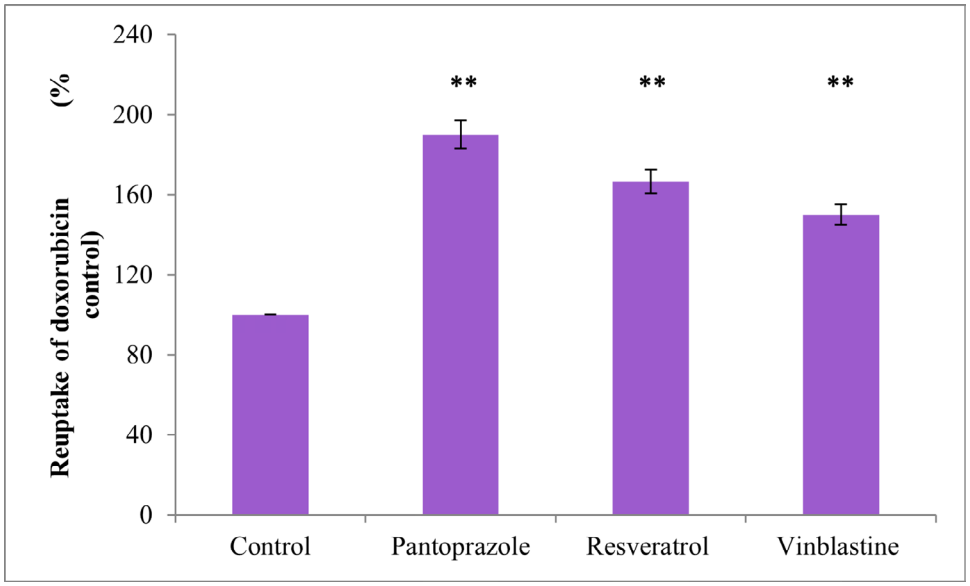

Figure 4: The Effect of BCRP Inhibitors (pantoprazole, resveratrol and vinblastine) on Doxorubicin Reuptake into Coca-2 Cells.

Discussion

Active transmembrane proteins known as efflux transporters (ABC) are widely expressed in the membranes of many tissues and organs, including the brain, gut, liver, and breast.[26] ABC transporters, like P-gp and BCRP, are intensively expressed in gastrointestinal epithelial membranes. These ABC efflux transporters have an influence on drugs oral bioavailability and absorption of a varied kind of pharmaceuticals, including chemotherapeutic treatments, and this phenomenon considerably increases MDR.[27] Short- term exposure to ABC inhibitors at the intestine cause modulation of transporter activity that could produce competitive or non-competitive action when another ABC proteins substrates were administrated.

Doxorubicin is intensively used anthracycline anti-cancer drug to manage many cancer types, though resistance amongst cancer cells has arisen as a chief barriers and problems to achieve an efficient treatment by using doxorubicin.[28]

In current study, the widely used in vitro model of everted gut (small intestine) and Coca-2 cells were utilized to examine the impacts of natural compounds such as resveratrol and vinblastine, along with BCRP inhibitors (pantoprazole), on the activity of ABC transporters related to doxorubicin. Initially, several tests had been used to ensure the feasibility and the functional activity of both everted gastrointestinal sacs and coca-2 cells were maintained during all the experiments.

First, investigations were conducted to examine the feasibility and the activity of the intestinal gut sacs throughout the experiment incubation time. Since most animals and humans absorb glucose mostly by active transport in small intestine, a glucose movment between the fluids within and outside sacs is thought to be an further indication of gut function.[29] The findings revealed that the glucose levels on the serosal side are markedly elevated compared to the mucosal side, suggesting that the everted sacs were functioning well besides, glucose was being well transported.

Additionally, until 60 minutes before the experiments ended, the activity of LDH enzyme, a sign of cell impairment,[30] remained constant at various time points. This finding suggests that gut sacs were unaffected and healthy until the incubation phase.[31]

The physiological function and activity of Coca-2 cell also examined throughout the experiment, the subculture of the cells did not effect on enzymes activity in Coca-2 cells including Glutathione Peroxidase, Superoxide Dismutase and Alkaline Phosphatase. In addition, by using MTT assay, the results had showed that there was no toxic effect of pantoprazole, doxorubicin, resveratrol and vinblastine on Coca-2 cell at the used concentrations. Current study concentrated on utilizing natural medical

product to rise the bioavailability and absorption of medications that have poor absorption because of ABC efflux transport. Pantoprazole is frequently utilized to investigate the inhibitory effect of other drugs as a standard inhibitor and as a BCRP substrate.[32] Since a BCRP transporter substrate, is a frequently used cancer treatment medication that has poor intestinal absorption.[8,33]

Initial study was conducted to approve that BCRP is extensively expressed in the everted gut. Using doxorubicin, resveratrol and vinblastine were significantly elevating pantoprazole penetration through the intestinal sac. Several studies approved that pantoprazole is a BCRP inhibitor[34,35] but no study examined the effect of BCRP on pantoprazole absorption.

To validate the functional activities of BCRP, more research was done. Intestinal everted sac treatment with the BCRP inhibitor pantoprazole results in a significant 1.8-fold increase in doxorubicin infiltration ($p \leq 0.001$). According to a previous study, doxorubicin and pantoprazole boost the availability of substrates in cancer cells while inhibiting the action of BCRP.[36] By using Coca-2 cell in this study, more evidence support that BCRP inhibition by pantoprazole significantly ($p \leq 0.001$) increase doxorubicin absorption in intestinal cell lines. Our findings are corroborated by another in vitro investigation, which showed that the combination of pantoprazole and doxorubicin greatly increased the penetration level of doxorubicin in solid tumours and multilayered cell cultures.[37] A study used tetrahydroisoquinoline derivatives which inhibit P-gp and BCRP and the results approved that doxorubicin passing the intestinal barrier in Caco-2 cells is increased by using ABC inhibitors.[38] Additionally, Studies results showed that pantoprazole improved the sensitivity of chemo-resistant oral epidermoid carcinoma cells (KB/V) to vincristine[39] and Pantoprazole increased methotrexate absorption by inhibiting BCRP in everted gut.[40] We think that no prior work carried out with an *in vitro* model of an everted gut to examine the impact of pantoprazole and doxorubicin together on each other's permeability.

Consequently, the current study inspected the effects of two medical natural substances, resveratrol and vinblastine on doxorubicin permeability. Resveratrol significantly decreased BCRP transporter activity (inhibition) and improve doxorubicin penetration from one side to other side through the gut sacs. The Coca-2 cells experiment showed a similar pattern as doxorubicin uptake significantly elevated in the presence of resveratrol. Another study approved that the treatment of Caco-2 cell with doxorubicin_and resveratrol, it increased the cell sensitivity to doxorubicin and enhanced it cytotoxicity by enhancing its penetration to the cells.[41]

Vinblastine effect on pantoprazole and doxorubicin permeability also are tested. The results showed that the above natural flavonoid product significantly improved pantoprazole and doxorubicin permeability proposing the inhibitory effect of it on BCRP, vinblastine reduce the efflux of both pantoprazole and doxorubicin by 1.5 fold in everted gut. It also increase doxorubicin reuptake by Coca-2 cells by 1.5 times. A research demonstrated that vinblastine had a low penetration through the Coca-2 cell line due to ABC efflux transporters.[42] Studies have been approved that vinblastine is a substrate of p-glycoprotein by using many cancer cells.[43,44] In addition, A research study showed that downregulation of mdr-1 gene (p-g gene) expression could increase vinblastine level and so its toxicity in HepG2 cells.[45] While there is no study test the effect of vinblastine on BCRP activity. We believe that there no research focused on studying the inhibitory effects of vinblastine on BCRP activity and doxorubicin permeability.

This is the first study demonstrated the inhibitory impact of vinblastine on BCRP-mediate transport of pantoprazole and doxorubicin.

This work uses an Everted gut model to show for the first time how these natural compounds affect the amount of BCRP in the gastrointestinal system.

Conclusion

It is well known that BCRP is one of the vital protein which cause a critical issue regarding anti-canser medication resistant. According to the results of the current study, employing natural products—especially common and safe natural herbs—could be a particularly helpful method to boost the penetration of several chemotherapy treatments such as doxorubicin and reduce resistant, that are substrates of ABC efflux transporters. In the future, other safe natural products with minimum toxicity might be investigated to increase the bioavailability of anti-cancer medications.

Conflict of Interest

The authors declare clearly there is no conflict of interest through other researchers.

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