Effect of Organic Isothiocyanates on the P-Glycoprotein- and MRP1-Mediated Transport of Daunomycin and Vinblastine

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Purpose. Organic isothiocyanates (ITCs), or mustard oils, are non-nutrient components present in the diet, especially in cruciferous vegetables. The purpose of this investigation was to examine the effect of ITCs on P-glycoprotein (P-gp)- and multidrug resistance-associated Protein (MRP1)-mediated transport in multidrug resistant (MDR) human cancer cell lines.

Methods. The direct effect of ITCs on the 2-h cellular accumulation of daunomycin (DNM) and vinblastine (VBL), substrates for both P-gp and MRP1, were measured in sensitive and resistant MCF-7 cells and in PANC-1 cells. Resistant MCF-7 cells (MCF-7/ADR) overexpress P-gp whereas PANC-1 cells overexpress MRP1. The following compounds were evaluated: allyl-, benzyl-(BITC), hexyl-, phenethyl-(PEITC), phenyl-, 1-naphthyl-(NITC), phenylhexyl-, phenylpropyl-, and phenylbutyl-ITC, sulforaphane, erucin, and erysolin.

Results. NITC significantly increased the accumulation of DNM and VBL in both resistant cell lines, but had no effect on DNM accumulation in sensitive MCF-7 cells. VBL accumulation in resistant MCF-7 cells was increased 40-fold by NITC whereas that in PANC-1 cells was increased 5.5-fold. Significant effects on the accumulation of DNM and VBL in resistant MCF-7 cells were also observed with benzyl-isothiocyanate whereas PEITC, erysolin, phenylhexyl-ITC, and phenylbutyl-ITC increased the accumulation of DNM and/or VBL in PANC-1 cells. Overall, the inhibitory activities of these compounds in MCF-7 cells and PANC-1 cells were significantly correlated ($r^2 = 0.77$ and 0.86 for DNM and VBL, respectively). Significant effects on accumulation were generally observed with the ITCs at 50 μM concentrations, but not at 10 μM concentrations.

Conclusions. One strategy to enhance the effectiveness of cancer chemotherapy is to reverse the MDR phenomena. Our results indicate that certain dietary ITCs inhibit the P-gp- and the MRP1-mediated efflux of DNM and VBL in MDR cancer cells and suggest the potential for diet-drug interactions.

KEY WORDS: multidrug resistance; phenethylisothiocyanate; benzylisothiocyanate; naphthylisothiocyanate; cancer chemotherapy.

INTRODUCTION

What may be considered a major setback from successful cancer chemotherapy is the phenomenon of simultaneous resistance to many structurally unrelated cytotoxic agents known as multidrug resistance (MDR; 1). One well-characterized mechanism is the overexpression of efflux proteins at the surface of the cell membrane, including p-glycoprotein (P-gp) and multidrug resistance–associated protein (MRP1). Overexpression of P-gp and/or MRP1 results in the increased efflux and therefore decreased intracellular concentrations of many natural product chemotherapeutic agents. These efflux pumps may be present at the time of diagnosis and/or may be overexpressed after drug exposure.

P-glycoprotein-mediated efflux is one mechanism of MDR that has been extensively studied. The 170kD P-gp encoded by the MDR1 gene belongs to the ATP-binding cassette (ABC) superfamily of proteins (ABCB1) and functions as an ATP-dependent efflux pump responsible for the transfer of a wide variety of xenobiotics and carcinogens from cells (2). The diverse classes of antitumor drugs that are P-gp substrates include anthracyclines, vinca alkaloids, epipodophyllotoxins, and taxanes. Besides being overexpressed in various tumor cells (3), P-gp is expressed endogenously in adrenal tissues, kidney, lung, liver, and colon (4). The differential expression of P-gp in normal tissues and its conservation among species suggest that the protein many have distinct physiologic roles associated with specialized cell functions. The tissue distribution of P-gp, mainly in the epithelia of excretory organs, and the ability to transport a wide range of lipophilic substrates, are compatible with the hypothesis that P-gp serves a detoxification function in the body. In cancer cells, the overexpression of P-gp decreases the intracellular concentrations of chemotherapeutic drugs and has been positively correlated with poor prognosis in cancers (2).

Overexpression of the 190-kd multidrug resistance-associated protein (MRP1) encoded by the MRP1 gene in cancer cells also results in MDR. Although first characterized in small cell lung cancer cells (5), MRP1 is present in almost all cells of the human body, as well as overexpressed in non-P-gp MDR cell lines of the lung, colon, gastric, ovary, and breast (6). MRP1 also belongs to the family of ABC membrane transporters (ABCC1), and in a similar manner as P-gp, mediates resistance to a range of structurally and functionally unrelated agents (7). However, whereas P-gp and MRP1 both transport a number of natural product chemotherapeutic agents, substrate preferences do exist. The preferred substrates for MRP1 are usually organic anions, in particular, drugs conjugated with glutathione (GSH), glucuronate, or sulfate. In fact, MRP acts as a GS-X pump, transporting drugs conjugated to GSH out of the cell (7).

The identification and characterization of these two efflux pumps in MDR has stimulated extensive research into the search for clinically useful inhibitors. Although many inhibitors including calcium channel blockers (e.g., verapamil, nifedipine), hypotensive drugs (reserpine), antibiotics (cephalosporins, gramicidin, puromycin), immunosuppressors (cyclosporinA and its derivatives), and many other lipophilic compounds have been identified and investigated, clinical trials have been largely unsuccessful as a result of dose-related toxicities that occur at the doses necessary to achieve MDR reversal (8).

The main objective of the present study was to examine the effects of dietary organic isothiocyanates (ITCs) on P-gp-

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and MRP1-mediated transport of chemotherapeutic agents in human cancer cell lines. Organic isothiocyanates (and glucosinolates, the biosynthetic precursors of ITCs in plants), also known as mustard oils, are widely distributed in edible plants, including cruciferous vegetables, with human consumption estimated at milligram quantities daily. Glucosinolate levels have been estimated to be as high as 180 mg/g of some vegetables (9). In the present investigation we examined the effects of a range of natural and synthetic ITCs on the cellular accumulation of the P-gp and MRP1 substrates, daunomycin (DNM) and vinblastine (VBL) after 2-h exposure times. Studies were performed in sensitive and resistant human breast cancer cells (MCF-7) and human pancreatic cancer cells (PANC-1). Resistant MCF-7 cells (MCF-7/ADR) overexpress P-gp whereas PANC-1 cells overexpress MRP1.

MATERIALS AND METHODS

Erysolin, phenyl ITC, β-phenylethyl ITC, α-naphthyl ITC, and verapamil were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Benzyl ITC, n-hexyl ITC, and allyl ITC were obtained from Aldrich (St. Louis, MO, USA). Sulforaphane and erucin were purchased from ICN (Aurora, OH, USA), and phenylpropyl ITC and phenybutyl ITC were purchased from LKT Laboratories (St. Paul, MN, USA). Phenylhexyl ITC was a gift from National Cancer Institute-Chemopreventive Division (Bethesda, MD, USA). Radiolabeled [^{3}H]-daunomycin (14.4 Ci/mmol) was purchased from NEN Life Science Products (Boston, MA, USA), and [^{3}H]-vinblastine sulfate (7.3 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA, USA). Cell culture reagents were supplied by GIBCO BRL (Buffalo, NY, USA), and cell culture flasks and dishes were purchased from Falcon (Becton Dickinson, Franklin Lakes, NJ, USA). Biodegradable liquid scintillation cocktail was purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Commassie blue dye reagent was obtained from Bio-Rad laboratory (Hercules, CA, USA). The MCF-7 and MCF-7/ADR cell lines were gifts from Dr. Ralph Bernacki (Roswell Park Cancer Institute). The PANC-1 cell line was obtained from American Type Culture Collection (Manassas, VA, UA). The monoclonal antibodies C219 and MRPr1 were obtained from Kamiya Biomedical Co. (Seattle, WA, USA).

Western Analysis of P-gp and MRP1

P-gp and MRP1 expression in the cells was determined by Western analysis using the antibodies C219 and MRPr1 as described previously (10). The protein molecular weight markers (Rainbow Markers, Amersham) used were myosin (200 kd), phosphorylase b (97.4 kd), and ovalbumin (46 kd). Membrane preparations from MCF-7 and PANC-1 cells were isolated using the method of Wils *et al.* (11). Protein concentrations were measured by the Bradford method (12) using a commercially available assay kit (Bio-Rad Labs) with γ-globulin as the standard. Proteins were electrophoresed on 7.5% SDS-polyacrylamide gels and electroblotted on nitrocellulose filter. The filter was blocked overnight at 4°C in Tris-buffered saline containing 0.2% (v/v) Tween 20 and 1% (w/v) bovine serum albumin, incubated with C219 (1 μg/mL) or MRPr1 (1:30) antibodies in blocking buffer for 2 h at room temperature. The filters were then washed in washing buffer (20 mM Tris base, 137 mM NaCl, 1% Tween 20, pH 7.6) and incubated with 1:1500 (v/v) anti-mouse IgG HRP secondary antibody (Amersham; for C219) or 1:1000 anti-rat IgG HRP secondary antibody (Zymed, San Francisco, CA, USA; for MRPr1), in blocking buffer for 2 h. After washing, the protein was detected using the ECL detection reagent (Amersham). Kodak 1D image analysis software was used to analyze the Western blot results.

Cell Culture

MCF-7 and MCF-7/ADR, used between passages 16–24, were grown in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, penicillin (10 units/mL), and streptomycin (10 μg/mL). Cells were incubated at 37°C supplemented with 5% CO_2/95% air. Cells were subcultured two to three times a week using 0.05% trypsin-0.53 mM EDTA. Cells were grown in 75-cm^2 plastic culture flasks that were seeded in 35-mm^2 plastic culture dishes for accumulation studies. Experiments were performed 2 to 3 days after seeding.

PANC-1 cells used between passages 60–75 were grown in Dulbecco's modified Eagle's medium supplemented with L-glutamine, sodium pyruvate, pyridoxine HCl, and 10% fetal bovine serum, which was maintained in an atmosphere of 10% CO_2/90% air at 37°C. Cells were subcultured every 2 to 3 days with 0.25% trypsin-2.6 mM EDTA. For experiments, cells were seeded on 35-mm^2 dishes at a density of 10^6 cells per dish and used 2 days later.

Accumulation Studies

Growth medium was removed from monolayer cells and cells were washed twice with sodium buffer (137 mM NaCl, 5.4 mM KCl, 2.8 mM $CaCl_2$, 1.2 mM $MgCl_2{\cdot}6H_20$, 10 mM HEPES, pH 7.4). One milliliter of incubation buffer containing 0.05 μM of [^{3}H]-DNM or 0.05 μM [^{3}H]-VBL and 100 μM of ITC was added to the dish and incubated for 2 h. Verapamil, a P-gp and MRP1 inhibitor, was used as a positive control in all studies. Concentration-dependent studies were performed with some of the ITCs using concentrations varying from 100 to 0.1 μM. The uptake was stopped by aspirating the incubation buffer and washing the cells three times with ice-cold stop solution (137 mM NaCl, 14 mM Tris-base, pH 7.4). One milliliter of 0.5% Triton-X-100 or 0.3 N NaOH-1%SDS was added to each dish, and aliquots were obtained after an hour. A liquid scintillation counter (1900 CA, Tri-Carb liquid scintillation analyzer, Packard Instruments Co.) was used to determine the radioactivity. The protein concentration was determined by the Bradford method (12) using a commercially available assay kit (Bio-Rad Labs) with γ-globulin as the standard.

Data Analysis

Statistical significance was determined by a one-way ANOVA followed by Dunnett's post hoc test. Differences were considered to be significant when $p < 0.05$.

RESULTS

MCF-7 Cells

Western Analysis

Western blot analyses were preformed to evaluate P-gp and MRP1 expression in MCF-7/WT, MCF-7/ADR, and

PANC-1 cells. There were undetectable amounts of P-gp in the MCF-7/WT and PANC-1 cell lines but high expression in the MCF-7/ADR cell line. PANC-1 cells showed high expression of MRP1. MCF-7/ADR cells also exhibited low expression of MRP1 (Fig. 1). The results found in this experiment confirmed those in the literature (13,14).

Time Course Study

The time course of uptake of 0.05 μM ^{3}H-DNM in the presence and absence of 100 μM verapamil, a typical inhibitor, was examined in sensitive (MCF-7/WT) and resistant (MCF-7/ADR) cells for up to 2 h (Fig. 2). For MCF-7/ADR cells, the accumulation of DNM was significantly greater in the presence of verapamil when compared with that in the absence of verapamil. In the sensitive cell line, which lacks P-gp, accumulation of DNM in the presence or absence of verapamil was unchanged; this demonstrates that verapamil influences the efflux of DNM through the inhibition of P-gp and not through other mechanisms in this cell line. Equilibrium conditions were achieved by 2 h in both the sensitive and resistant MCF-7 cells.

DNM Accumulation

The effect of various organic ITCs on DNM accumulation was examined in MCF-7/WT cells (Fig. 3). Verapamil did not significantly increase DNM accumulation in the sensitive cells. Only phenylpropyl ITC and phenylhexyl ITC produced significant increases in DNM accumulation in these cells. In MCF-7/ADR cells, verapamil was able to significantly increase DNM accumulation by 2.5-fold compared with the control. Few ITCs were found to inhibit the efflux of DNM, with the most active compound being 1-naphthylisothiocyanate (NITC), which increased DNM accumulation by 4-fold; benzylisothiocyanante (BITC) produced an effect that was similar in magnitude to that of verapamil 100 μM. All other compounds did not significantly alter DNM accumulation. Concentration-dependent studies demonstrated significant activity for NITC at concentrations of 50 μM but not at 10 μM (results not shown).

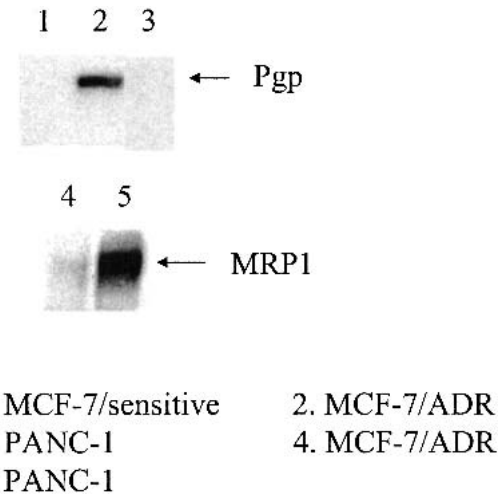

Fig. 1. Western blots of P-gp and MRP1 in MCF-7, MCF-7/ADR, and PANC-1 cells, using the antibodies C219 and MRPr1, respectively (as described in the Materials and Methods section).

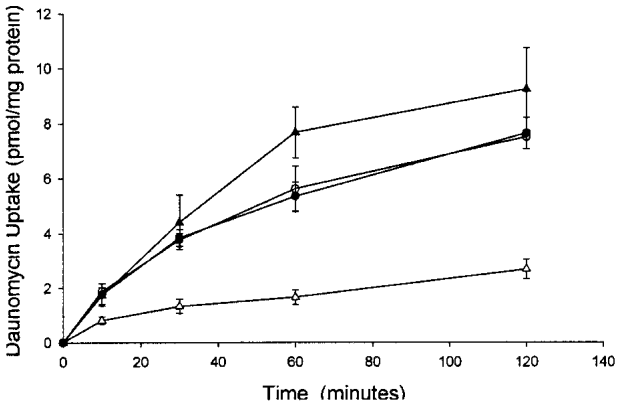

Fig. 2. Time course of daunomycin uptake in MCF-7 sensitive and resistant cells. DNM (0.05 μM) uptake was measured in the presence and absence of verapamil. (▲) MCF-7/ADR + verapamil (100 μM), (△) MCF-7/ADR control, (●) MCF-7/WT + verapamil (100 μM), (○) MCF-7/WT control. Data are mean ± SD of data from one representative study. The study was repeated with similar results.

VBL Accumulation

The uptake of VBL was examined in the presence and absence of ITCs. In MCF-7/ADR cells, verapamil signifi-

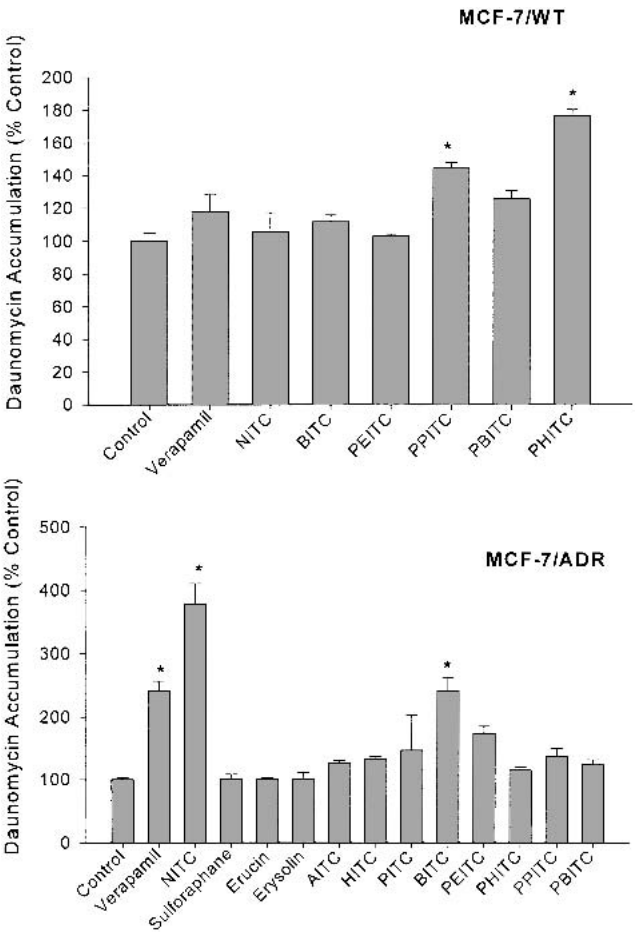

Fig. 3. Effect of organic isothiocyanates (ITCs) on daunomycin accumulation in MCF-7 cells. The 2-h accumulation of 0.05 μM daunomycin was measured in the presence of various ITCs (100 μM). Control represents the uptake in the absence of ITCs. Each bar represents mean ± SE, n = 9–12, *p < 0.001.

cantly increased the accumulation of VBL by 33-fold, phenylhexyl ITC by 10-fold, and NITC by 40-fold (Fig. 4). The greatest effects of the ITCs on accumulation were seen for VBL in MCF-7/ADR cells.

PANC-1 Cells

DNM Accumulation

In PANC-1 cells, phenethylisothiocyanate (PEITC), erysolin, NITC, and verapamil were able to significantly increase DNM accumulation (Fig. 5). A number of other ITCs, including BITC, allyl ITC, and hexyl ITC, demonstrated a trend towards increased accumulation of DNM ($p < 0.1$). Concentration-dependent studies demonstrated significant activity for NITC and PEITC at 50 μM concentrations but not at 10 μM concentrations (results not shown).

VBL Accumulation

Verapamil was able to significantly increase VBL accumulation by 4-fold. The ITCs that demonstrated significant effects were: NITC (5.5-fold), PEITC (2-fold), phenylhexyl ITC (3-fold), and phenylbutyl ITC (2.5-fold). All other compounds did not have significant effects, although a number showed a trend towards significance, including BITC, allyl ITC, and hexyl ITC (Fig. 6). The correlation between ITC inhibition (percent control values) for DNM and VBL in PANC-1 cells had an r^2 value of 0.37 ($p < 0.05$; not shown.)

We also examined the correlation between ITC inhibition in MCF-7 cells and PANC-1 cells. The ITC-mediated changes in cellular accumulation for both DNM and VBL in MCF-7/ADR and PANC-1 cells were highly correlated with r^2 values of 0.77 for DNM ($p < 0.05$; Fig. 7A) and 0.86 for VBL ($p < 0.005$; Fig. 7B).

DISCUSSION

Drug resistance represents a major cause for therapeutic failure and death in cancer treatment. An important mechanism of this resistance is the enhanced cellular efflux of a wide variety of structurally distinct classes of chemotherapeutic agents because of the overexpression of P-gp and/or MRP1. Studies of biopsy samples from patients have revealed elevated levels of *P*-gp in tumors of every histologic type, with a strong association in leukemias, lymphomas, and some childhood solid tumors between the detection of tumor P-gp and poor response to therapy (15). MRP1 has been identified in a number of different cancers (16): in neuroblastoma, MRP1 levels are elevated and are significantly correlated with N-myc, a negative prognostic factor for response to chemotherapy in neuroblastoma patients. Buser *et al.* (17) reported a high prevalence of P-gp in breast cancer tumor tissue: 83% in early breast cancer and 100% in primarily metastatic breast cancer. One strategy for reversing MDR in cancer has been

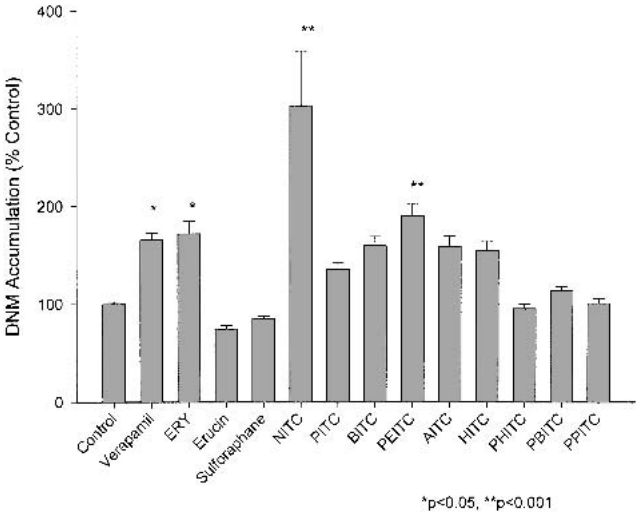

Fig. 5. Effect of organic isothiocyanates (ITCs) on daunomycin accumulation in PANC-1 cells. The 2-h accumulation of 0.05 μM daunomycin was measured in the presence of various ITCs (100 μM). Control represents the uptake in the absence of ITCs. Each bar represents mean ± SE, n = 9–12, *p < 0.05 **p < 0.001.

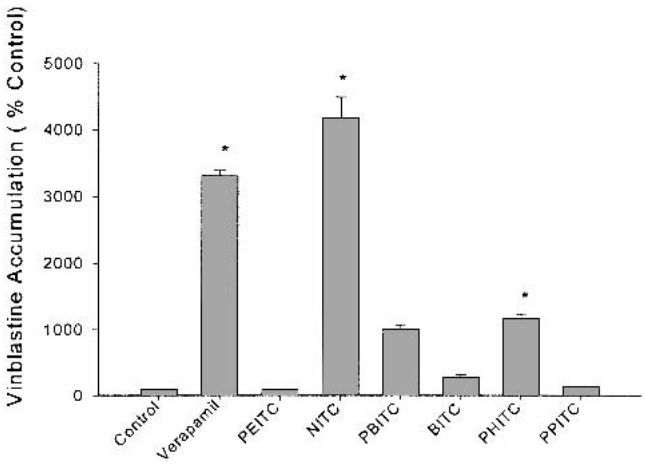

Fig. 4. Effect of organic isothiocyanates (ITCs) on vinblastine accumulation in MCF-7/ADR cells. The 2-h accumulation of 0.05 μM vinblastine was measured in the presence of various ITCs (100 μM). Control represents the uptake in the absence of ITCs. Each bar represents mean ± SE, n = 9–12, *p < 0.001.

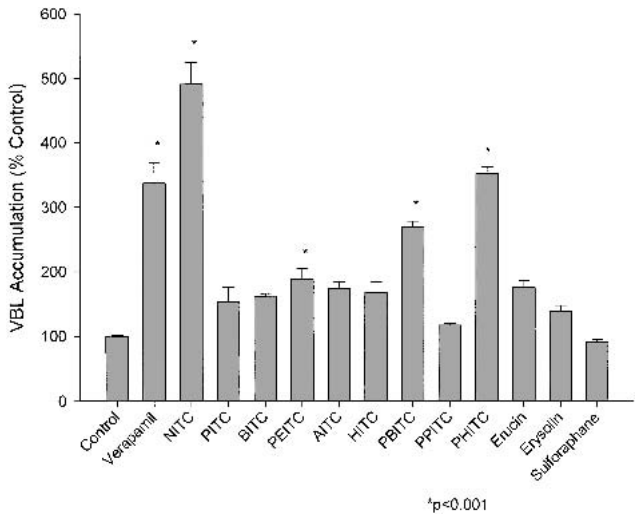

Fig. 6. Effect of organic isothiocyanates (ITCs) on vinblastine accumulation in PANC-1 cells. The 2-h accumulation of 0.05 μM vinblastine was measured in the presence of various ITCs (100 μM). Control represents the uptake in the absence of ITCs. Each bar represents mean ± SE, n = 9–12, * p <0.001.

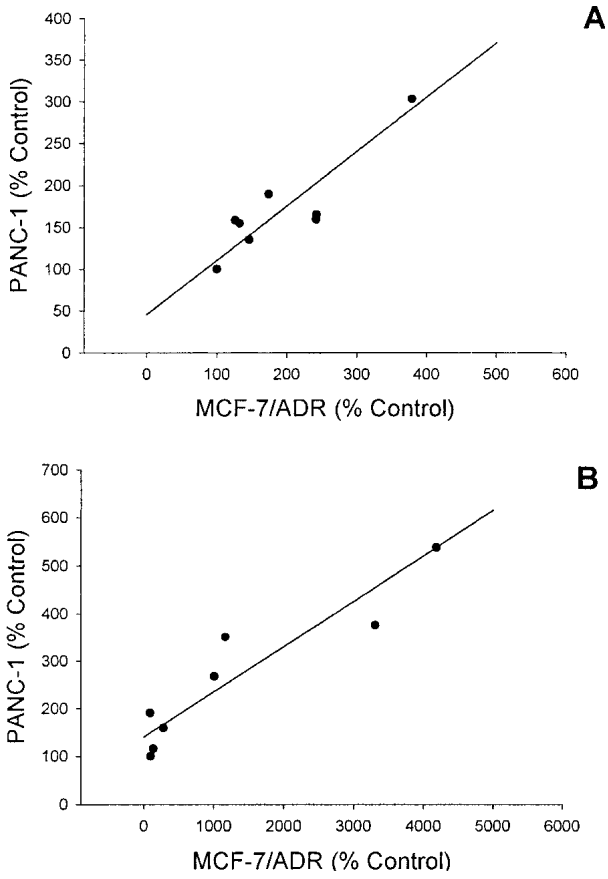

Fig. 7. Correlation between ITC inhibition in MCF-7/ADR cells and PANC-1 cells. (A) The relationship between ITC inhibition of daunomycin in MCF-7/ADR cells with that in PANC-1 cells. $r^2 = 0.77$, $p < 0.05$. (B) The relationship between ITC inhibition of vinblastine in MCF-7/ADR cells with that in PANC-1 cells. $r^2 = 0.86$, $p < 0.005$.

the concomitant use of chemical agents that are by themselves nontoxic but that increase the accumulation of chemotherapeutic drugs in MDR cells through the inhibition of P-gp- or MRP1-mediated efflux of these agents.

In this study, we investigated a class of dietary compounds, the organic ITCs, as inhibitors of P-gp- and MRP1-mediated drug resistance in cancer cell lines. The organic ITCs are components present in the diet, especially in cruciferous vegetables such as broccoli, watercress, cabbage, and brussel sprouts. Numerous experiments have reported that ITCs can inhibit tumor formation of the skin, lung, colon, and breast in animal models (9,18,19), although the mechanism by which this happens is still not completely understood. ITCs are currently being evaluated in clinical trials for the prevention of lung cancer (19). There is substantial evidence that the inhibition of tumorigenesis is partly the result of the direct inhibition and/or downregulation of the CYP-450s responsible for carcinogen activation (16). In addition, ITCs can induce phase II enzymes responsible for the detoxification of electrophilic intermediates formed during phase I metabolism (19). Other mechanisms are likely involved in the chemopreventive effects of ITCs: recent studies have indicated that sulforaphane induces cell cycle arrest and apoptosis in HT29 human colon cancer cells (20) and PEITC also induces apoptosis in cells (21).

Although the organic isothiocyanates represent a group of lipophilic natural products, they have not previously been investigated as substrates or inhibitors of P-gp or MRP1. We have found that NITC and BITC can increase the accumulation of DNM and VBL in the drug-resistant human breast cancer cell line MCF-7 without affecting accumulation in sensitive MCF-7 cells. Interestingly, two of the ITCs tested, phenylpropyl ITC and phenylhexyl ITC, significantly increased the accumulation of DNM in the MCF-7/WT cells but not in the MCF-7/ADR cells. The mechanism underlying this interaction is unknown. Additionally, a number of organic ITCs, including NITC and PEITC, increased the 2-h accumulation of DNM and VBL in PANC-1 cells, which overexpress MRP1 but not P-gp. At this time, it is not known whether these compounds represent substrates for P-gp or MRP1 or whether they are only inhibitors. Because the effects occur rapidly, this suggests that the inhibition might involve a direct interaction at the binding site or at an allosteric site that affects the binding of DNM or VBL. P-gp has been reported to have more than one substrate-binding site. Shapiro and Ling (22) reported that P-gp contains three distinct sites for drug binding, one which transports rhodamine 123, a second that transports Hoechst 33342, and a third that is specific for prazosin or progesterone (23). The anthracyclines inhibit rhodamine 123 transport and stimulate Hoechst 33342 transport whereas VBL, actinomycin D, and etoposide inhibit transport of both dyes. This suggests that compounds like DNM may represent a substrate for only one site whereas VBL may be a substrate for more than one site.

Substrates for MRP1 are endogenous and exogenous organic anions that are conjugated by glutathione, glucuronide, or sulfate, including leukotriene C4 (cysteinyl leukotrienes), glutathione disulfide (oxidized glutathione), and steroid glucuronides (17β-estradiol 17-β-D-glucuronide; 7). Natural product chemotherapeutic agents that do not form a glutathione conjugate, such as anthracyclines, vinca alkaloids, methotrexate, fluorouracil, and chlorambucil (24) are also substrates for MRP1. These drugs are likely transported by MRP1 in a GSH-dependent manner, which may involve the cotransport of GSH and the chemotherapeutic agent (24). Dietrich *et al.* (25) have demonstrated the MRP2-mediated biliary excretion of NITC, either as a GSH conjugate or in association with GSH, indicating that it is a substrate for MRP2. Our studies have demonstrated that the inhibitory effects of the ITCs on either DNM or VBL accumulation in MCF-7/ADR and PANC-1 cells are highly correlated. This finding was not unexpected because there is overlap in substrate specificity for these transporters, with many of the natural product chemotherapeutic agents being substrates for both transporters.

Our concentration-dependent studies indicate that the ITCs are not potent direct inhibitors of P-gp- or MRP1-mediated efflux. Concentrations of 50 μM of NITC, PEITC, and BITC are effective inhibitors; after a 2-h accumulation study, the compounds were ineffective at a concentration of 10 μM. However, concentration-dependent effects after prolonged exposures have not been examined. After vegetable consumption, concentrations of ITCs in plasma are likely in the nM range (26), although there have been no studies that have determined blood levels of unchanged ITCs. Blood concentrations of ITCs would be expected to vary because of genetic differences in their metabolism by glutathione-S-

transferase M1 and T1 (GSTM1 and GSTT1). Conjugation with glutathione, followed by further conjugation reactions to form the mercapturic conjugate, represents the major route of elimination of PEITC and BITC. GSTM1 and T1 exhibit genetic polymorphisms: 60% of Chinese subjects and 40–50% of people in a variety of ethnic groups are deficient in the GSTM1 gene whereas 10–30% of Europeans are deficient in the GSTT1 polymorphism (27,28). These subjects would be expected to have higher blood concentrations of ITCs than those with the wild-type enzyme. It has been reported that the protective effect of dietary ITC intake for lung cancer risk among current smokers is greatest in individuals null for both GSTM1 and GSTT1 genotypes (27,28). What might be more relevant than plasma ITC concentrations would be intracellular concentrations. Intracellular concentrations of ITCs have been reported to be much higher than extracellular concentrations: for example, cells exposed to 100 μM concentrations of sulforaphane have intracellular concentrations of 6.4 mM, likely as GSH conjugates (29). The relationship between intracellular concentrations and efficacy has not been evaluated for the ITCs.

P-gp and MRP1 also play important roles in the bioavailability, distribution, and elimination of administered drugs (8). In the kidney, P-gp is highly expressed on the brush border of the proximal renal tubule. Speeg *et al.* (30) have demonstrated the inhibition of renal clearance of colchicine by cyclosporin, suggesting that MDR modulators may alter the renal elimination processes of anticancer drugs by blocking P-gp in kidneys. P-gp and some isoforms of MRP are present in the apical membrane of intestinal epithelial cells, where they can limit the absorption of xenobiotics, and in the canalicular membrane of hepatocytes, where they can affect biliary excretion. For example, oral administration of paclitaxel to wild-type and *mdr1a* knockout mice resulted in a 6-fold higher plasma level of paclitaxel in the latter, at least partly as a result of increased bioavailability (31). We found that 100 μM concentrations of NITC, BITC, and PEITC could significantly increase the 2-h accumulation of DNM in the porcine renal cell line LLC-PK1, which expresses low levels of P-gp (Tseng E and Morris ME, unpublished results). Whether this is the result of inhibition of P-gp and/or other transporters is currently unknown. It is likely that exposure to ITCs present in the diet may affect the bioavailability, and possibly disposition, of compounds transported by P-gp and/or MRP1.

The results of this investigation demonstrate for the first time that P-gp and MRP1 activity can be modulated by naturally occurring organic ITCs. Further studies are needed to evaluate the time-dependent nature of this inhibition, and its clinical relevance.

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