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Dossier : Anticancer compounds and drug resistance circumvention

Role of xenobiotic efflux transporters in resistance to vincristine

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Abstract

This study characterized interactions between efflux transporters (P-glycoprotein (MDR1) and multidrug resistance associated proteins (MRPs1−3)) and vincristine (VCR), using cell lines with differential transporter expression, and studied effects of P-glycoprotein inhibition on VCR transport and toxicity. Caco2 (express MDR1, MRPs 1−3), LS174T (express MDR1, MRPs 1, 3), and A549 (express MRPs 1−3) cells were used. To study VCR transport (effective permeability, P_{eff}), VCR (1−500 nM) was added to the donor chambers of permeable supports containing Caco2 monolayers, and receiving chamber concentrations were measured. Cytotoxicity experiments were conducted with escalating concentrations of VCR in all cell lines. To determine the contribution of MDR1, experiments were also conducted with LY335979, a specific MDR1 inhibitor. VCR P_{eff} was 2×10^{-6} cm/s in Caco2 cells. LY335979 increased P_{eff} in a dose dependent manner (up to 7-fold with 1 μM LY335979) in Caco2 cells. Caco2 and LS174T cell viability decreased significantly when co-incubated with both VCR and LY335979 (1 μM) ($P < 0.05$), however this was not observed in A549 cells. In summary, MDR1 plays an important role in VCR efflux; MDR1 inhibition increased VCR P_{eff} in Caco2 cells, and increased VCR cytotoxicity in Caco2 and LS174T cells (both express MDR1), but not A549 cells (minimal MDR1 expression). Inhibition of MDR1 may be a viable strategy to overcome VCR resistance in tumors expressing MDR1, however the presence of other efflux transporters should also be considered, as this will influence the success of such strategies.

Keywords: Vincristine; Transporters; P-glycoprotein; MDR1; Multidrug resistance associated proteins

1. Introduction

Resistance to pharmacological therapy is problematic in the treatment of numerous types of malignancies. One of the most important reasons for cancer chemotherapy resistance is the increased expression of xenobiotic transporters that mediate the efflux of anticancer agents from cancer cells [1]. The ATP-binding cassette (ABC) transporters actively transport xenobiotics across cell membranes. To date, 49 members have been identified in the ABC family. Of these, four transporters, ABCB1 (P-glycoprotein (P-gp, MDR1)), ABCC1 (multidrug resistance associated protein 1 (MRP1)), ABCC2 (multidrug resistance associated protein 2 (MRP2) or canalicular multispecific organic anion transporter (cMOAT)) and ABCC3 (multidrug resistance associated protein 3 (MRP3)), are the most prominent drug efflux transporters, and their functional expression has been associated with tumor response and outcome for a number of different cancers [2–4].

Vincristine (VCR) is a vinca alkaloid that is widely used in the treatment of acute leukemias and solid tumors. It has been observed that cancer cells can develop resistance toward VCR [5]. This phenomenon may be mediated, in part, by drug efflux transporters, including MDR1, however the relative contribution of specific transporters is unclear [4,6–9]. Similarly, the role of MDR1 inhibition to overcome VCR resistance in cell lines expressing multiple transporters with overlapping substrate affinities is also unclear. The objectives of the study described herein were to characterize the role of MDR1 (P-glycoprotein) in VCR transport and toxicity, and to investigate the role of P-glycoprotein inhibition in overcoming resistance to VCR.

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2. Methods

2.1. Materials

Caco2 cells (a human intestinal carcinoma cell line), LS174T cells (a human intestinal carcinoma cell line), and A549 cells (a human lung carcinoma cell line), were obtained from American Type Culture Collection (ATCC, Manassas, VA). Dulbecco's modified Eagle's medium (DMEM, containing 4.5 g/L glucose and L-glutamine), non-essential amino acid, HEPES, penicillin-G/streptomycin, sodium pyruvate, fetal bovine serum (FBS), PBS, trypsin-EDTA and F-12K nutrient mixture (Kaighn's modification) were purchased from GIBCO® Invitrogen (Carlsbad, CA). Sterile collagen-coated permeable cell culture inserts (Transwell®, pore size 3 μm) were manufactured by Corning Costar (Corning, NY). VCR and radiolabeled VCR ([^{3}H]VCR) were purchased from Sigma Chemical Co. (St. Louis, MO). LY335979, a MDR1 specific inhibitor, was a generous gift from Eli Lily and Company (Indianapolis, IN). ScintiSafe Econo 1 cocktail was from Fisher Scientific (Fairlawn, NJ). All other chemicals and reagents were of the highest grade.

2.2. Cell culture

Caco2 (express MDR1, MRPs 1–3), LS174T (express MDR1, MRP 1,3), and A549 (express MRPs 1–3) cells were used in experiments; previous work in our laboratory has characterized the differential transporter expression in these cells (Table 1) [10]. Caco2 cells were grown with DMEM supplemented with 1% non-essential amino acids, 1% HEPES, 100 U/mL penicillin-G/streptomycin, 1% sodium pyruvate and 10% FBS. LS174T cells were grown in DMEM supplemented with 10% FBS. A549 cells were grown with F-12K nutrient mixture (Kaighn's modification) supplemented with 10% FBS. All cells were grown at 37 °C in a humidified 5% CO_2 atmosphere. Media was changed every 48 h and cells were passaged once weekly at approximately 80% confluence by trypsinization. Passages 20–30, 170–180, and 120–130 were used for Caco2, LS174T and A549 experiments, respectively.

2.3. Transport of VCR in Caco2 cells

In order to characterize the actions of drug transporters on VCR, directional transport assays were performed as described previously with modification [11,12]. Twenty-one days prior to experiments, Caco2 cell suspensions (100 μL 1×10^5 cells/mL) were added to the apical sides of cell culture inserts. On the day of experiments, transepithelial electrical resistance (TEER) was measured in each well using a epithelial ohmmeter (World Precision Instruments Inc., Sarasota, FL); wells registering a resistance of 200–300 Ω-cm^2 were used in the transport experiments. Prior to the start of the transport experiments (1–2 h), the medium in each compartment was replaced with serum-free medium.

Table 1
Relative *MDR1*, *MRP1*, *MRP2* and *MRP3* gene mRNA expression in human immortalized cell lines (Caco2, LS174T, and A549 cells) [10]

	Origin	*Mdr1*	*Mrp1*	*Mrp2*	*Mrp3*
Caco2	Human intestinal carcinoma	+++	++	+++	++
LS174T	Human intestinal carcinoma	+++	+++	+	++
A549	Human lung carcinoma	+	+++	+++	++

Various concentrations of [^{3}H]VCR (1–500 nM) were applied to the donor sides of the cell culture supports. Cells were then incubated for pre-determined time periods (0–180 min). Aliquots (100 μL) were taken from basolateral compartments for apical-to-basolateral experiments at different sampling times. In basolateral-to-apical transport experiments, 50 μL aliquots were obtained from apical side at 120 min. The sampling times for apical-to-basolateral transport were 15, 30, 60, 120, 180 min. Sample aliquots (100 μL from basolateral chambers and 50 μL from apical chambers) were added to 10 mL of ScintiSafe Econo 1 cocktail and were analyzed using a Tri-Carb 2100 TR liquid scintillation analyzer (Packard Instrument Company, Meriden, CT).

Directional VCR transport experiments were also carried out in the presence of the MDR1 specific inhibitor, LY335979. LY335979 (0.05, 0.1, 0.5, 1 μM (in sera free media with 0.1% MeOH)) was added to both apical and basolateral chambers prior to the additional of VCR. After incubation for 45 min at 37 °C, medium was aspirated from both chambers. [^{3}H]VCR (100 or 500 nM) along with various concentrations of LY335979 was applied to the donor chamber of the inserts. Sampling times and procedures were the same as those in VCR directional transport assays. VCR accumulation was recorded as disintegrations per minute (dpm). The effective permeability was obtained as described previously:

$$P_{\text{eff}} = \frac{\mathrm{d}C}{\mathrm{d}t}\frac{V}{C_0 \mathrm{Ar}}$$

where V is the volume of the receiver chamber, C_0 is the initial drug concentration in the donor chamber, Ar is the surface area of the membrane that separates chambers A and B, and $\mathrm{d}C/\mathrm{d}t$ is the permeability rate (the slope of the plot of concentration in the receiver chamber versus time) [13,14].

2.4. Cell viability tests

The viability of each of the three cell lines in the presence or absence of 1 μM LY335979 was assessed using CellTiter 96 AQueous One solution from Promega (Madison, WI). Briefly, at the time of passage, cell suspensions (100 μL 1×10^5 cells/mL) were added into the wells of 96-well plates. After 24 h stabilization, medium was replaced by either 100 μL of fresh serum free medium or 1 μM LY335979 (in serum free medium with 0.1% MeOH) and incubated at 37 °C for 60 min. At the end of 60 min, treatment was started by adding 100 μL of different concentrations of VCR solutions or mixture of different concentrations of VCR together with 1 μM LY335979. Treatment conditions used in these experiments were: control (blank