Multiple Efflux Pumps Are Involved in the Transepithelial Transport of Colchicine: Combined Effect of P-Glycoprotein and Multidrug Resistance-Associated Protein 2 Leads to Decreased Intestinal Absorption Throughout the Entire Small Intestine

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ABSTRACT:
The purpose of this study was to thoroughly characterize the efflux transporters involved in the intestinal permeability of the oral microtubule polymerization inhibitor colchicine and to evaluate the role of these transporters in limiting its oral absorption. The effects of P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP) inhibitors on colchicine bidirectional permeability were studied across Caco-2 cell monolayers, inhibiting one versus multiple transporters simultaneously. Colchicine permeability was then investigated in different regions of the rat small intestine by in situ single-pass perfusion. Correlation with the P-gp/MRP2 expression level throughout different intestinal segments was investigated by immunoblotting. P-gp inhibitors [N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide (GF120918), verapamil, and quinidine], and MRP2 inhibitors [3-[[3-[2-(7-chloroquinolin-2-yl)vinyl]phenyl]-2(dimethylcarbamoyethyl)sulfanyl]methylsulfanyl] propionic acid (MK571), indomethacin, and p-aminobenzoic acid (p-AH)] significantly increased apical (AP)-basolateral (BL) and decreased BL-AP permeability. An overall increase in efflux transport dominated the intestinal permeability process.

ATP-dependent efflux transporters, such as P-glycoprotein [(Pgp) ABCB1], multidrug resistance-associated protein 2 (MRP2, ABCC2), and breast cancer resistance protein (BCRP, ABCG2), have been shown to play significant roles in drug absorption, distribution and clearance processes and in drug-drug and drug-food interactions (Dahan and Altman, 2004; Raub, 2006; Takano et al., 2006; Miller et al., 2008). These transporters are present in many biological membranes, including the villus tip of the apical brush-border membrane of gut enterocytes and actively cause efflux of drugs from gut epithelial cells back into the intestinal lumen. P-gp recognizes a variety of structurally and pharmacologically unrelated neutral and positively charged compounds (Mizuno et al., 2003; Raub, 2006). MRP2 transports relatively hydrophilic molecules, including the glucuronide, glutathione, and sulfate conjugates of endogenous and exogenous compounds (Suzuki and Sugiyama, 2002). BCRP recognizes relatively hydrophilic anticancer agents (Doyle and Ross, 2003). Because of the overlapping substrate specificities, multiple efflux pumps might be involved in limiting the membrane permeability of a drug molecule. Drug substances that have been shown to be substrates for several efflux pumps include atorvastatin (Lau et al., 2006), rosuvastatin (Kitamura et al., 2008), cyclosporine (Mannermaa et al., 2006), sulfasalazine (Dahan and Amidon, 2009c), saquinavir (Usansky et al., 2008), pantoprazole. P-gp/MRP2 inhibitors combinations greatly reduced colchicine mucosal secretion, including complete abolishment of efflux (GF120918/MK571). Colchicine displayed low (versus metoprolol) and constant permeability along the rat small-intestine. GF120918 significantly increased colchicine permeability in the ileum with no effect in the jejunum, whereas MK571 augmented jejunal permeability without changing the ileal transport. The GF120918/MK571 combination caused an effect similar to that of MK571 alone in the jejunum and to that of GF120918 alone in the ileum. P-gp expression followed a gradient increasing from proximal to distal segments, whereas MRP2 decreased from proximal to distal small intestinal regions. Overall, it was revealed that the combined effect of P-gp and MRP2, but not BCRP, dominates colchicine transepithelial transport, leading to complete coverage of the entire small intestine, and makes the efflux transport dominate the intestinal permeability process.

ABBREVIATIONS: P-gp, P-glycoprotein; MRP2, multidrug resistance-associated protein 2; BCRP, breast cancer resistance protein; ER, efflux ratio; BL, basolateral; AP, apical; GF120918, N-[4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl]-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide; MK571, 3-[[3-[2-(7-chloroquinolin-2-yl)vinyl]phenyl]-2(dimethylcarbamoyethyl)sulfanyl]methylsulfanyl] propionic acid; p-AH, p-aminobenzoic acid; FTC, fumitremorgin C; MES, 4-morpholineethanesulfonic acid; HPLC, high-performance liquid chromatography; DMEM, Dulbecco’s modified Eagle’s medium; TEER, transepithelial electrical resistance; TBS-T, Tris-buffered saline with Tween 20.
Colchicine (Fig. 1) is an oral microtubule polymerization inhibitor, prescribed in gout therapy (Ben-Chetrit and Levy, 1998; Terkeltaub, 2003), for prevention of acute attacks of familial Mediterranean fever (Dinarello et al., 1974; Amital and Ben-Chetrit, 2004), and in the treatment of immune and inflammatory diseases [primary biliary cirrhosis (Kaplan and Gershwin, 2005) and systemic scleroderma (Alarcon-Segovia et al., 1979)]. Colchicine is metabolized in the liver, mainly by demethylation mediated by cytochrome P450 3A4 (Leighton et al., 1990; Tateishi et al., 1997). In addition, colchicine is susceptible to P-gp-mediated efflux transport, and increased oral absorption and pharmacodynamic effects due to P-gp inhibition were reported (Dintaman and Silverman, 1999; Bittner et al., 2002). These barriers are considered to contribute to its low and variable absorption: colchicine absolute oral bioavailability is in the range of 40% (Rochdi et al., 1994; Ferron et al., 1996). We have recently studied the pharmacokinetic interaction of colchicine with grapefruit juice and showed that the juice augments colchicine intestinal absorption through a P-gp inhibition mechanism (Dahan and Amidon, 2009a). However, in all P-gp inhibition experiments, an efflux ratio (ER) \( P_{BL-AP}/P_{AP-BL} \) of 1 could not be achieved, indicating that efflux transporters other than P-gp may be involved in the intestinal absorption process of colchicine, presumably contributing to its low oral bioavailability.

The purpose of the present study was to thoroughly characterize the contribution of efflux transporters to the intestinal permeability process of colchicine after oral administration. The effects of various P-gp, MRP2, and BCRP inhibitors on the bidirectional transcellular permeability of colchicine were studied across Caco-2 cell monolayers, inhibiting one transporter at a time versus multiple transporters simultaneously. The effective permeability of colchicine was then investigated in different regions of the rat small intestine by the in situ single-pass intestinal perfusion model, in the presence versus absence of P-gp (GF120918) and MRP2 (MK571) inhibitors, again, one at a time versus simultaneous inhibition. These results were then correlated to the expression level of these transporters throughout the different intestinal segments, measured by Western blot analysis. Overall, this setup allowed us to confirm the role of the different efflux pumps involved in colchicine intestinal permeability.

### Materials and Methods

**Materials.** Colchicine, metoprolol, phenol red, verapamil, quinidine, indomethacin, \( p \)-aminophenolic acid (\( p \)-AH), probenecid, fumitremorgin C (FTC), lucifer yellow, MES buffer, glucose, CaCl\(_2\), MgCl\(_2\), and trifluoroacetic acid were purchased from Sigma-Aldrich (St. Louis, MO). Pantoprazole, potassium chloride, and NaCl were obtained from Thermo Fisher Scientific (Waltham, MA). GF120918 was generously donated by GlaxoSmithKline Inc. (Research Triangle Park, NC), MK571 was purchased from Alexis Biochemicals (Lausen, Switzerland). Acetonitrile and water (Acros Organics, Geel, Belgium) were HPLC grade. Physiological saline solution was purchased from Hospira Inc. (Lake Forest, IL). All other chemicals were of analytical reagent grade.

**Cell Culture.** Caco-2 cells (passages 25–32) from American Type Culture Collection (Manassas, VA) were routinely maintained in Dulbecco’s modified Eagle’s medium (DMEM) (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum, 1% nonessential amino acids, 1 mM sodium pyruvate, and 1% l-glutamine. Cells were grown in an atmosphere of 5% CO\(_2\) and 90% relative humidity at 37°C. The DMEM was routinely replaced by fresh medium every 3 days. Cells were passaged upon reaching approximately 80% confluence using 4 ml of trypsin-EDTA (Invitrogen).

**Caco-2 Permeability Studies.** Transcellular transport studies were performed using a method described previously (Gao et al., 2001; Dahan et al., 2009a). In brief, \( 5 \times 10^5 \) cells/cm\(^2\) were seeded onto collagen-coated membranes (12-well Transwell plate, 0.4-μm pore size, 12 mm diameter; Costar, Cambridge, MA) and were allowed to grow for 21 days. Mannitol and lucifer yellow permeabilities were assayed for each batch of Caco-2 monolayers (\( n = 3 \)), and TEER measurements were performed on all monolayers (Millicell-ERS epithelial voltohmeter; Millipore Corporation, Billerica, MA). Monolayers with apparent mannitol and lucifer yellow permeability \(<3 \times 10^{-7} \) cm/s and TEER values \( >300 \) Ω cm\(^2\) were used for all studies. On the day of the experiment, the DMEM was removed, and the monolayers were rinsed and incubated for 20 min with a blank transport buffer. The transport buffer contained 1 mM CaCl\(_2\), 0.5 mM MgCl\(_2\), 145 mM NaCl, 3 mM KCl, 1 mM NaH\(_2\)PO\(_4\), 5 mM d-glucose, and 5 mM MES. A similar pH was used in both apical and basolateral sides (pH 6.5) to maintain a constant degree of ionization in both AP-BL and BL-AP direction experiments and to avoid the possible influence of this factor on the permeability across the cells. After the 20-min incubation, the drug-free transport buffer was removed from the donor side (apical in the AP-BL direction or basolateral in the BL-AP direction studies) and was replaced by colchicine uptake buffer solution (pH 6.5), with or without inhibitors. Throughout the experiment, the transport plates were kept in a shaking incubator (50 rpm) at 37°C. Samples were taken from the receiver side at various time points up to 120 min (100 μl from basolateral side or 70 μl from apical side), and similar volumes of blank buffer were added after each sample withdrawal. At the last time point (120 min), samples were taken from the donor side also to confirm mass balance. Samples were immediately assayed for drug content. All Caco-2 monolayers were checked for confluence by measuring the TEER before and after the transport study.

**Inhibition Experiments.** The concentration-dependent effects of the P-gp inhibitors GF120918 (5 and 10 μM), verapamil (10, 50, and 100 μM), and quinidine (10, 50, and 100 μM), as well as the MRP2 inhibitors MK571 (10, 50, and 100 μM), indomethacin (10, 50, and 100 μM), \( p \)-AH (1, 5, and 10 mM), and probenecid (10, 50, and 100 μM), and the BCRP inhibitors FTC (5, 10, and 20 μM) and pantoprazole (10, 50, and 100 μM) on the bidirectional transport of colchicine (0.1 mM) across Caco-2 cell monolayers were examined. The results of these experiments were evaluated in comparison with the bidirectional transport of 0.1 mM colchicine in the absence of inhibitors. The effects of the following combinations of P-gp and MRP2 inhibitors on colchicine bidirectional transport were then investigated: GF120918 and MK571 (10 and 100 μM, respectively), quinidine and MK571 (100 μM), verapamil and MK571 (100 μM), quinidine and indomethacin (100 μM), and verapamil and indomethacin (100 μM). The combination that produced the highest efflux inhibition, GF120918 and MK571, was then selected for further analysis in rats.

**Single-Pass Intestinal Perfusion Studies in Rats.** All animal experiments were conducted using protocols approved by the University Committee of Use and Care of Animals, University of Michigan, and the animals were housed and handled according to the University of Michigan Unit for Laboratory Animal Medicine guidelines. Male albino Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 250 to 280 g were used for all perfusion studies. Before each experiment, the rats were fasted overnight (12–18 h) with free access to water. Animals were randomly assigned to the different experimental groups.

The procedure for the in situ single-pass intestinal perfusion followed...
previously published reports (Kim et al., 2006; Dahan et al., 2009b). In brief, rats were anesthetized with an intramuscular injection of 1 ml/kg ketamine-xylazine solution (9%:1%, respectively) and placed on a heated surface maintained at 37°C (Harvard Apparatus Inc., Holliston, MA). The abdomen was opened by a midline incision of 3 to 4 cm. A proximal jejunal segment (3-cm average distance of the inlet from the ligament of Treitz) or a distal ileal segment (3-cm average distance of the outlet from the cecum) of approximately 10 cm was carefully exposed and cannulated on two ends with flexible polyvinyl chloride tubing (2.29 mm i.d., inlet tube 40 cm, outlet tube 20 cm; Thermo Fisher Scientific). Care was taken to avoid disturbing the circulatory system, and the exposed segment was kept moist with 37°C normal saline solution. All solutions were incubated in a 37°C water bath. The isolated segment was rinsed with blank perfusion buffer, pH 6.5, at a flow rate of 0.5 ml/min to clean out any residual debris.

At the start of the study, perfusion solution containing colchicine (0.1 mM), 10 mM MES buffer, pH 6.5, 135 mM NaCl, 5 mM KCl, and 0.1 mg/ml phenol red with an osmolarity of 290 mOsmol/l, with or without the different inhibitors (10 μM GF120918 and 100 μM MK571), was perfused through the intestinal segment (Watson Marlow Pumps 323S; Watson-Marlow Bredel Inc., Wilmington, MA), at a flow rate of 0.2 ml/min. Phenol red was added to the perfusion buffer as a nonabsorbable marker for measuring water flux. Metoprolol was coperfused with the colchicine as well, as a compound with known permeability that serves as a marker for the integrity of the experiment and as a reference standard for permeability in close proximity to the low/high permeability class boundary (Kim et al., 2006). The perfusion buffer was first perfused for 1 h, to assure steady-state conditions (as also assessed by the inlet over outlet concentration ratio of phenol red, which approaches 1 at steady state). After reaching steady-state, samples were taken at 10-min intervals for 1 h (10, 20, 30, 40, 50, and 60 min). All samples including perfusion samples at different time points, original drug solution, and inlet solution taken at the exit of the syringe were immediately assayed by HPLC. After the termination of the experiment, the length of each perfused intestinal segment was accurately measured.

Preparation of Rat Brush Border Homogenates for the Analysis of P-gp and MRP2 Expression Along the Small Intestine. The small intestine of male Wistar rats, starting from the ligament of Treitz and ending at the entrance to the cecum, was removed and rinsed with ice-cold physiological saline solution. The intestine was cut into five symmetrically spaced equal segments, 10-cm-long each, and placed in phosphate-buffered saline containing 1.5 mM EDTA, 0.5 mM dithiothreitol, pH 7.4, and protease inhibitor cocktail (Invitrogen). The intestinal segments were placed on an ice-cold glass surface, and the mucosa was gently squeezed out and placed in similar buffer containing 0.25 M sucrose. Intestinal segments were homogenized with a Polytron homogenizer at 25,000 rpm (three 10-s periods), followed by ultrasonication (three 10-s periods) on ice. The homogenates were centrifuged at 900 g for 10 min, and the supernatant was carefully removed and used for protein content analysis (DC protein assay; Bio-Rad Laboratories, Hercules, CA) and immunoblotting.

Immunoblot Analysis of P-gp, MRP2, and BCRP. Immunoblot analysis of P-gp, MRP2, BCRP, and villin was performed using a method described previously with some modifications (MacLean et al., 2008). From each sample, 100 μg of protein were resolved with 10% SDS-polyacrylamide gel electrophoresis (Invitrogen) followed by electrophoretic transfer onto Hybond ECL nitrocellulose membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Membranes were blocked overnight in TBS-T solution containing 3% bovine serum albumin at 4°C, followed by incubation with monoclonal anti-P-gp antibody (C219, 1:200 dilution in TBS-T; Zymed Laboratories, South San Francisco, CA), monoclonal anti-MRP2 antibody (M106 III-6, 1:100 dilution in TBS-T; Alexis Biochemicals), monoclonal anti-BCRP antibody (BXP-21, 1:200 dilution in TBS-T; Alexis Biochemicals), or monoclonal anti-villin antibody (H-60, 1:400 dilution in TBS-T; Santa Cruz Biotechnology Inc., Santa Cruz, CA) for 2 h at room temperature. The membranes were then
incubated for 1 h at room temperature with alkaline phosphatase-conjugated goat anti-mouse IgG (1:3000 dilution; Santa Cruz Biotechnology Inc.). The blots were subsequently visualized with enhanced chemifluorescence substrate using a Typhoon 9200 Variable Mode Imager (GE Healthcare, Buckinghamshire, UK), and the protein bands of interest were quantified using ImageQuant 5.2 software (GE Healthcare). P-gp and MRP2 expression levels were first normalized to the corresponding villin expression, and then, within each set of samples (segments 1–5 from the same rat), the lowest intensity band was set to a value of 1, and the intensities of the other four segments were set relative to that one.

**Data Analysis.** The permeability coefficient (P_app) across Caco-2 cell monolayers was calculated from the linear plot of drug accumulated in the absorptive (AP-BL) direction, according to the following equation:

\[
P_{\text{app}} = \frac{1}{C_{0}A} \times \frac{dQ}{dt}
\]

where \(dQ/dt\) is the steady-state appearance rate of the drug on the receiver (serosal in the case of AP-BL studies or mucosal in the case of BL-AP studies) side, \(C_{0}\) is the initial concentration of the drug in the donor side, and \(A\) is the monolayer growth surface area (1.12 cm²). Linear regression was carried out to obtain the steady-state appearance rate of the drug on the receiver side (\(R^2 > 0.99\) in all experimental groups).

The ER (i.e., the net efflux of colchicine) was determined by calculating the ratio of P_app in the secretory (BL-AP) direction divided by P_app in the absorptive (AP-BL) direction, according to the following equation:

\[
\text{ER} = \frac{P_{\text{app,BL-AP}}}{P_{\text{app,AP-BL}}}
\]

**Statistical Analysis.** All Caco-2 experiments were performed in triplicate (unless stated otherwise), and all animal experiments were done in each experimental group.

Where \(Q\) is the perfusion buffer flow rate, \(C_{\text{in}}/C_{\text{out}}\) is the ratio of the outlet concentration and the inlet or starting concentration of the tested drug that has been adjusted for water transport, \(R\) is the radius of the intestinal segment (set to 0.2 cm), and \(L\) is the length of the intestinal segment.

The net water flux in the single-pass intestinal perfusion studies, resulting from both water absorption and efflux in the intestinal segment, was determined by measurement of phenol red, a nonabsorbed, nonmetabolized marker. The phenol red (0.1 mg/ml) was included in the perfusion buffer and co-perfused with the tested drugs. The measured \(C_{\text{out}}/C_{\text{in}}\) ratio was corrected for water transport according to the following equation:

\[
\frac{C_{\text{out},\text{phenol red}}}{C_{\text{in},\text{phenol red}}} = \frac{C_{\text{out}}}{C_{\text{in}}} \times \frac{C_{\text{in}}}{C_{\text{out},\text{phenol red}}}
\]

where \(C_{\text{in,phenol red}}\) is equal to the concentration of phenol red in the inlet sample, and \(C_{\text{out,phenol red}}\) is equal to the concentration of phenol red in the outlet sample.

**Analytical Methods.** The amount of colchicine in the Caco-2 medium and the simultaneous analyses of colchicine, metoprolol, and phenol red in the rat perfusion buffer were performed using a HPLC system (2695 Separation Module; Waters, Milford, MA) with a photodiode array UV detector (Waters 2996). Samples were filtered (Unifilter 96-well microplate 0.45-µm filters; Whatman, Florham Park, NJ), and Caco-2 medium aliquots of 50 µl were injected into the HPLC system. The HPLC conditions were as follows: XTerra, RP18, 3.5 µm, 4.6 × 100 mm column (Waters); a gradient mobile phase, going from 90:10 to 50:50% v/v aqueous/organic phase, respectively, over 15 min; the aqueous phase of 0.1% trifluoroacetic acid in water and organic phase of 0.1% trifluoroacetic acid in acetonitrile; and flow rate of 1 ml/min at room temperature. The detection wavelengths were 275, 265, and 350 nm, and the retention times were 6.5, 9.5, and 12.0 min for metoprolol, phenol red, and colchicine, respectively.
among the experimental groups, the nonparametric Kruskal-Wallis test was used for multiple comparisons, and the two-tailed nonparametric Mann-Whitney U test was used for two-group comparison where appropriate. \( p < 0.05 \) was termed significant.

**Results**

**Effect of Different Efflux Transporters on Colchicine Transcellular Permeability Across Caco-2 Cell Monolayers.** The flux of colchicine across Caco-2 monolayers in the AP-BL and BL-AP directions, in the presence versus absence of different concentrations of P-gp or MRP2 inhibitors, is presented in Figs. 2 and 3, respectively. All P-gp inhibitors (GF120918, verapamil, and quinidine) significantly increased AP-BL and decreased BL-AP direction transport, in a concentration-dependent manner. Among these inhibitors, GF120918 showed the most potent effect; however, none of these inhibitors produced an efflux ratio of 1, i.e., complete inhibition of the drug efflux. Likewise, the MRP2 inhibitors MK571, indomethacin, and \( p \)-AH displayed a concentration-dependent decrease in colchicine mucosal secretion, in both AP-BL and BL-AP directions. Again, an efflux ratio of 1 could not be achieved. Among these inhibitors, MK571 showed the most potent effect. Probeneicid, a MRP2 inhibitor, showed some inhibition of colchicine BL-AP transport but no effect on the AP-BL direction.

The effects of the BCRP inhibitors FTC and pantoprazole on colchicine bidirectional transport across Caco-2 cell monolayers is presented in Fig. 4. It can be seen that colchicine transepithelial permeability was not affected by the inhibition of BCRP. The Caco-2 expression of P-gp, MRP2, and BCRP was validated using Western blot analysis (Fig. 5), confirming that these efflux transporters were indeed present in the cell culture experiments in the protein level.

The effects of different combinations of P-gp and MRP2 inhibitors on the bidirectional Caco-2 transport of colchicine are presented in Fig. 6, and the resulting efflux ratios of colchicine from the different inhibition experiments are summarized in Fig. 7. All P-gp and MRP2 inhibitors combinations were able to greatly reduce colchicine mucosal secretion, and the combination of the two most potent inhibitors, GF120918 and MK571, abolished colchicine efflux transport completely. Hence, this combination was chosen for further evaluation in rats.

**Effect of P-gp and MRP2 on Colchicine Intestinal Permeability Along the Rat Small Intestine.** Colchicine \( P_{eff} \) values obtained after in situ perfusion to the rat proximal jejunum or distal ileum, in the presence versus absence of the P-gp inhibitor GF120918 and the MRP2 inhibitor MK571, are presented in Fig. 8. Without inhibitors, colchicine displayed low (relative to metoprolol) and constant permeability in the proximal and distal segments of the rat small intestine.
FIG. 6. The effect of different P-gp/MRP2 inhibitors combinations on colchicine flux (0.1 mM) across Caco-2 cell monolayers in the absorptive (AP-BL) and secretory (BL-AP) directions. Data are presented as the mean ± S.D.; n = 3 in each experimental group.

FIG. 7. ERs (P_{BL-AP}/P_{AP-BL}) obtained for colchicine transport across Caco-2 cell monolayers in the different inhibition experiments (highest inhibitor concentration). Data are presented as the mean ± S.D.; n = 3 in each experimental group. PPZ, pantoprazole; VER, verapamil; QUIN, quinidine; PROB, probenecid; INDO, indomethacin.
mental groups (2.4E-5 in each group; GF120918 and MK571 simultaneously. Data are presented as the mean ± S.D.; n = 4 in each group; **, p < 0.01. Metoprolol permeability was similar in all experimental groups (2.4E-5 ± 0.4E-5; n = 12).

Segmental-dependent colchicine permeability was obtained in the presence of the efflux transporter inhibitors; inhibition of P-gp significantly increased the permeability in the ileum with no effect on the jejunal transport, whereas MRP2 inhibition augmented colchicine permeability in the jejunum, with no change in the ileal P$_{eff}$. When both inhibitors were simultaneously coperfused with colchicine, comparable permeability in the proximal and distal segments of the rat small intestine was obtained; the GF120918/MK571 combination caused an effect similar to that of MK571 alone in the jejunum and to that of GF120918 alone in the ileum.

**P-gp and MRP2 Protein Expression Levels Along the Rat Small Intestine.** Figure 9 shows representative Western blots of rat mucosal samples from different segments along the small intestine, obtained with monoclonal antibodies against P-gp, MRP2, and villin. A semi-quantitative analysis of the efflux protein bands, normalized to villin, is presented in Fig. 10. Significant regional-dependent P-gp and MRP2 expression levels were found throughout the rat small intestine, with the two transporters exhibiting gradients of opposing trends; P-gp expression levels increased from the proximal to the distal segments, whereas MRP2 levels decreased from the proximal to the distal small intestinal regions.

**Discussion**

Segmental-dependent expression levels of different transporters, both influx and efflux, have significant implications for oral drug delivery. With regard to efflux transporters, we recently showed that the low expression of P-gp in the proximal regions of the small intestine leads to a minimal role for this transporter in the absorption of P-gp substrates from these segments, enabling a window for such drug compounds to be relatively well absorbed after oral administration (Dahan and Amidon, 2009b). The same phenomenon, in the opposite direction, may be obtained for MRP2 substrate drugs. In this article, we demonstrate the combined effect of P-gp and MRP2 on a drug substance that is a substrate for both P-gp- and MRP2-mediated efflux. This powerful “collaboration” leads to complete coverage of the entire small intestinal length and makes the efflux transport dominate the intestinal permeability process. The data presented in this article can explain colchicine low and variable oral absorption and reveal potential drug interactions.

P-gp-mediated efflux of colchicine has been demonstrated before, both by us (Dahan and Amidon, 2009a) and others (Dintamani and Silverman, 1999; Troutman and Thakker, 2003; Niel and Scherrmann, 2006). However, an efflux ratio of 1 could not be achieved under inhibition of P-gp alone, indicating the involvement of efflux transporters other than P-gp in its intestinal absorption process. Hence, we investigated the role of MRP2 and BCRP in colchicine bidirectional permeability. The MRP2 inhibitor MK571 exhibited a strong concentration-dependent increase in AP-BL transport, accompanied by a decrease in BL-AP transport, illustrating, for the first time, that colchicine is susceptible to MRP2-mediated efflux transport (Fig. 3). We were surprised to find that probenecid, which is a nonspecific inhibitor of MRP2, had little effect on the BL-AP transport, and no effect on the AP-BL direction. A similar phenomenon was reported before for fluvastatin, where although the involvement of MRP2 in the intestinal absorption and biliary secretion of this HMG-CoA reductase inhibitor was evident, probenecid showed neither in vitro nor in vivo effects on its transport (Lindahl et al., 2000, 2004). This finding may be explained by a relatively poor affinity of probenecid for MRP2 compared with the drug investigated. Indeed, probenecid was reported to have higher affinity for MRP1 than for MRP2 and to be less effective as an MRP2-mediated efflux inhibitor than indomethacin (Bakos et al., 2000). The nonspecific MRP2 inhibitors indomethacin and p-AH displayed a concentration-dependent increase in AP-BL and a decrease in BL-AP transport, further supporting the involvement of this transporter in the colchicine efflux process. The specific BCRP inhibitors FTC and pantoprazole had no effect on colchicine bidirectional transport across Caco-2 monolayers (Fig. 4), indicating that this efflux pump is not involved in the colchicine intestinal permeability process. Overall, it was revealed that the combined effect of P-gp and MRP2, but not BCRP, mediates the transepithelial efflux transport of colchicine. This was further evident by the experiments involving a combination of P-gp and MRP2 inhibitors (Fig. 6): all P-gp and MRP2

![FIG. 8. $P_{eff}$ (cm/s) values obtained for colchicine after in situ single-pass intestinal perfusion to the proximal jejunum and to the distal ileum of the rat, without inhibitors, in the presence of GF120918 or MK571 and in the presence of both GF120918 and MK571 simultaneously. Data are presented as the mean ± S.D.; n = 4 in each group; **, p < 0.01. Metoprolol permeability was similar in all experimental groups (2.4E-5 ± 0.4E-5; n = 12).](https://dmd.aspetjournals.org/doi/10.1124/dmd.109.029042)

![FIG. 9. Analysis of P-gp, MRP2, and villin levels in different segments along the rat small intestine. Segments 1 to 5 correspond to equal, 10-cm-long, symmetrically spaced segments, starting from the ligament of Treitz (segment 1) and ending at the entrance to the cecum (segment 5). P-gp was probed with the monoclonal antibody C219, MRP2 was probed with the monoclonal antibody M. III-6, and villin was probed with the monoclonal antibody H-60. Data shown are representative preparations from three animals.](https://dmd.aspetjournals.org/doi/10.1124/dmd.109.029042)
Colchicine plasma levels after oral administration are expected to be proportional to the fraction of drug absorbed, and hence changes in colchicine plasma levels may be the result of altered P-gp/MRP2 function. This analysis highlights the fact that the significance of the data presented in this article goes beyond contributing to a better understanding of the mechanisms behind colchicine low oral bioavailability, but may also be relevant to the prediction of different clinical scenarios, e.g., side effects or drug-drug interactions. Although the focus of this work is the intestinal absorption process after oral administration, our data may be relevant to other colchicine pharmacokinetic processes, e.g., distribution and clearance. Indeed, decreased colchicine biliary clearance from 0.122 to 0.024 ml/min/kg by cyclosporine through P-gp inhibition has been reported (Speeg et al., 1992), leading to increased colchicine plasma levels, and to a clinically relevant interaction. Because MRP2 is expressed on the canalicular membrane and plays an important role in the biliary excretion of various drugs, the fact that colchicine is a MRP2 substrate as revealed in this article may point out new mechanism-based drug-drug interactions. Because colchicine is a drug with a narrow therapeutic index and severely toxic side effects (effective steady-state plasma concentrations range from 0.5 to 3 ng/ml with toxic effects appearing at approximately 3 ng/ml) (Ferron et al., 1996), this may be a most relevant issue.

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