Multidrug Resistance Gene G1199A Polymorphism Alters Efflux Transport Activity of P-Glycoprotein

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ABSTRACT

The significance of the human multidrug resistance gene (MDR1) G1199A polymorphism, resulting in a Ser400Asn modification in P-glycoprotein (P-gp), remains unclear. We have developed stable recombinant LLC-PK1 epithelial cells expressing either MDR1wt or MDR11199 to evaluate functional consequences of G1199A [N-(4-2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isouquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide]. P-gp activity observed in MDR1wt and MDR11199 cells was completely inhibited in the presence of the specific P-gp inhibitor GF120918. Comparable expression of mRNA and protein in the MDR1-expressed cells and correct localization of P-gp in the apical membrane of recombinant cells was verified. Mean intracellular rhodamine-123 (R123) accumulation, measured by flow cytometry, was approximately 4.75-fold higher in MDR11199 recombinant cells than MDR1wt cells. Cytotoxicity studies have shown that MDR1wt and MDR11199 cells exhibited similar resistance, as measured by EC50 values, to doxorubicin (155 ± 68 versus 120 ± 32 nM); however, MDR11199 cells were more resistant to vinblastine (1.41 ± 0.51 versus 15.7 ± 4.0 nM; p < 0.001) and vincristine (1.18 ± 0.56 versus 3.41 ± 1.47 nM; p < 0.05). The apparent transepithelial permeability ratios of R123 in MDR1wt and MDR11199 cells were 3.54 ± 0.94 and 2.02 ± 0.51 (p < 0.05), respectively. Therefore, the G1199A polymorphism alters the efflux and transepithelial permeability of a fluorescent substrate and sensitivity to select cytotoxic agents, which may influence drug disposition and therapeutic efficacy of some P-gp substrates.

The human multidrug resistance gene (MDR1) encodes a 170-kDa integral membrane protein that mediates ATP-dependent substrate efflux. The protein product, P-glycoprotein (P-gp), a member of the ATP-binding cassette superfamily of transporters, resides in the plasma membrane and functions as an efflux transporter of a variety of natural compounds and lipophilic xenobiotics (for review, see Lin, 2003). Although the contribution of P-gp in multidrug resistance for cancer chemotherapy is well documented, the role of P-gp in drug disposition is not fully understood and has continued to generate significant debate. P-gp mediates the energy-dependent efflux of a broad range of xenobiotics in epithelial tissues throughout the human body, including the intestinal mucosa, liver canalicular membrane, kidney proximal tubules, blood-brain barrier, and placenta (Schinkel, 1997). P-gp efflux may, therefore, act to decrease intestinal absorption, enhance biliary excretion and renal tubular secretion, and limit drug distribution to the fetus and brain. Because P-gp is found in tissues important in drug disposition, variation in expression and function of P-gp due to genetic polymorphisms of MDR1 may influence pharmacokinetics and, in turn, pharmacodynamics.

Recent progress has been made in identifying genetic polymorphisms in the MDR1 gene in normal human tissues. The first major screen of the MDR1 gene in 188 healthy Caucasian subjects, identified 15 single nucleotide polymorphisms; however, only one, a C → T transition at nucleotide position 3435 (C3435T), was shown to correlate with decreased intestinal P-gp expression and digoxin exposure in vivo (Hoffmeyer et al., 2000). Because the C3435T polymorphism in exon 26 is a synonymous polymorphism that does not modify the amino acid sequence of P-gp, several investigators have searched for clues to the significance of C3435T. Another study reported that C3435T is linked to a nonsynonymous G2677T polymorphism, resulting in an alanine-to-serine transition at amino acid 893, and another synonymous SNP, C1236T (Kim et al., 2001). Several other studies have at-

ABBREVIATIONS: MDR1, multidrug resistance gene; P-gp, P-glycoprotein; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; BSA, bovine serum albumin; HBSS, Hanks’ balanced salt solution; PE, phycoerythrin; R123, rhodamine-123.

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tempted to link MDR1 polymorphisms, particularly C3435T, to changes in P-gp expression and function, and subsequent changes in drug disposition profiles (Hitzl et al., 2001; Sakaeda et al., 2001; Tanabe et al., 2001; von Ahsen et al., 2001; Calado et al., 2002; Drescher et al., 2002; Fellay et al., 2002; Gerloff et al., 2002; Goto et al., 2002; Horinouchi et al., 2002; Kurata et al., 2002; Min and Ellingrod, 2002; Moriya et al., 2002; Nakamura et al., 2002; Pauli-Magnus et al., 2002; Siegmund et al., 2002; Oselin et al., 2003; Verstuyft et al., 2003). However, much of the data has been contradictory and inconclusive as to the influence of MDR1 SNPs and haplotypes on P-gp expression and in vivo drug disposition. A systematic study, designed to address genetic variants at the cellular and molecular level, is needed to define the functional significance of and the linkage between genetic polymorphisms of MDR1 and their impact on clinical pharmacokinetics.

Therefore, we have developed a recombinant expression system in epithelial cells capable of expressing P-gp variants in a reproducible manner to systematically study the influence of genetic polymorphisms in MDR1. Thus far, in vitro studies in the literature to evaluate the influence of MDR1 polymorphisms on P-gp efflux have produced variable results. Discrepancies exist in the reported influence of G2677T and C3435T on P-gp activity in various vector and expression systems (Kim et al., 2001; Kimchi-Sarfaty et al., 2002; Morita et al., 2003). In addition, some transient expression systems used to evaluate changes in activity cannot be used to evaluate transepithelial transport, which is important in assessing drug uptake and permeability. Because linkage to C1236T and G2677T complicates the influence of C3435T, we have instead chosen to evaluate the functional role of the G1199A polymorphism as a model for our recombinant P-gp system in epithelial cells capable of expressing P-gp variants and haplotypes on P-gp expression as well as altering the sensitivity profiles of cells to cytotoxic drugs.

**Materials and Methods**

**Cell Culture.** LLC-PK1 control and transfected cells were grown in complete media consisting of RPMI 1640 medium (Invitrogen, Carlsbad, CA) supplemented with 10% (v/v) fetal calf serum and 1% (v/v) antibiotic-antimycotic and grown at 37°C in the presence of 5% CO₂. For deconvolution immunoﬂuorescent microscopy and transepithelial transport studies, cells were grown in Medium 199 (Invitrogen) supplemented with l-glutamine, 10% (v/v) fetal calf serum, and 1% (v/v) antibiotic-antimycotic and grown at 37°C in the presence of 5% CO₂.

**Generation of MDR1wt and MDR11199 Plasmids.** Total RNA was extracted from the MDR1-overexpressing cell line, MES-SA-DX5, and full-length MDR1 cDNA was generated using the high-fidelity protocol developed previously (Yang et al., 2002). The isolated MDR1 cDNA was cloned into a linearized pCR3.1 TA vector (Invitrogen) containing cytomegalovirus and T7 promoters capable of transcription, also described previously (Yang et al., 2002). The plasmid stock was designated as MDR1wt-type (MDR1wt).

The plasmid containing the G1199A polymorphism was generated by subcloning a fragment containing the polymorphism into the MDR1wt plasmid using the restriction enzyme HindIII. This strategy employed two PCR steps to insert the 1199A variant and is further detailed in Fig. 1. Two pairs of primers were designed with an online primer design software program (Primer3) based on an MDR1 sequence available in the GenBank database (accession no. M14758). The first pair overlapped the G1199A region with the 1199A variant in the middle of the oligonucleotide: 5' CAGAAAATGGTCTCAGTCAATTTACC2CATCGAAAAG 3' (residues 1182–1216; forward primer) and 5'TTTCGAGATGGGTAC3GAAGTAAGGCATCTG 3' (residues 1182–1214; reverse primer). The other set of primers overlapped the restriction enzyme sites for HindIII, one recognition site in the pCR3.1 vector, 5' GTTTAACCCTGAAGTCTACC 3' (residues 697–764; forward primer), and one site in MDR1 cDNA, 5' GGTACTAAGCTTCGACCTGGTTAC 3' (residues 2031–2052; reverse primer). The first PCR step generated two fragments containing 1199A at one end of the fragment and the HindIII site at the other end. The two fragments were annealed together and amplified using the HindIII primers. The amplified PCR fragment and the pCR3.1-hMDR1 plasmid were digested with HindIII and annealed together. Clones were screened by restriction enzyme mapping with PstI and EcoRI and the sequence was verified using Big-Dye 3.0 chemistry (Applied Biosystems, Foster City, CA) and an ABI Prism 377 DNA Sequencer (Applied Biosystems). The variant plasmid stock was designated MDR11199.

**Isolation of Stable Recombinant Cells Expressing MDR1wt or MDR11199.** The MDR1wt and MDR11199 plasmids were transfected into the porcine kidney epithelial cell line LLC-PK1. Ten million cells were resuspended in complete media and transferred to a 0.4-cm electroporation cuvette (Bio-Rad, Hercules, CA) with 10 µg

![Fig. 1. Schematic representation of the generation of the MDR11199 plasmid. The MDR11199 plasmid was generated by introducing a 1199A variant into the original MDR1wt plasmid. The first step in generating the MDR11199 plasmid used the HindIII and 1199A primer sets to generate two fragments each containing 1199A at one end and a HindIII site at the other from the original MDR1wt plasmid. Next, the fragments were annealed and amplified using the HindIII primer set. Finally, the fragment containing 1199A and the pCR3.1-hMDR1 vector were digested with HindIII and religated. The MDR11199 plasmid was validated by restriction enzyme and sequencing analyses. MDR1wt and MDR11199 plasmids contain T7 and cytomegalovirus promoters and ampicillin and neomycin resistance genes.](image-url)
of plasmid DNA (either MDR1_b or MDR1_c). Electroporation was performed at 230 V and 975 μF in a Gene Pulser II (Bio-Rad). Cells were plated on ice for 10 min and cultured in complete media for selection. Transfected LLC-PK1 cells with the highest level of P-gp expression were selected to generate stable cell lines expressing MDR1_b or MDR1_c. Cells were treated for 3 days with 300 μg/ml G418, a neomycin derivative (Calbiochem, San Diego, CA). A fraction of the cells were briefly exposed for another 3 days to 5 μM doxorubicin (VHA PLUS; VHA Inc., Irving, TX), a cytotoxic P-gp substrate, and continued selection by G418 pressure. The cells expressing high levels of P-gp were further expanded and verified to have stable expression, allowing for functional evaluation.

**Characterization of P-gp Expression.** Absolute quantitation of MDR1 mRNA transcripts in cellular samples was performed using the ABI Prism 7900HT sequence detection system (Applied Biosystems). An MDR1 RNA standard was generated by the method described previously, and concentration was measured by absorbance at 260 nm and converted to the number of copies of MDR1 RNA by the molecular weight (Yang et al., 2002). A dilution series of the MDR1 RNA standard was used to generate a standard curve of the number of copies of MDR1 mRNA versus Ct value. After RNA isolation, 100 ng of total RNA of each cellular sample was analyzed in triplicate to obtain a Ct value and estimation of the number of copies of MDR1 mRNA.

Immunoblot analysis was used to detect protein expression. Briefly, 2 × 10^5 cells were pelleted and lysed in a lysis buffer containing SDS and β-mercaptoethanol. Protein concentration was measured by a microplate assay protocol (Bio-Rad). The P-gp-positive control is derived from the MESA-SD-X5 cell line known to highly express P-gp. Electrophoresis and transfer was run according to instructions for Mini-PROTEAN II Electrophoresis Cell and Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). Nitrocellulose blots were blocked with 5% evaporated milk in 0.1% Tween 20, 20 mM Tris-HCl, 0.9% NaCl, pH 7.6, buffer. Immunoblotting was performed with the anti-P-gp monoclonal antibody F4 (Sigma-Aldrich, St. Louis, MO) followed by a secondary horseradish peroxidase-conjugated goat anti-mouse IgG. Enhanced chemiluminescence reagents were used as a substrate and blots were exposed to X-ray film for visualization of the protein bands.

Cell surface expression of P-gp was analyzed by flow cytometry. Briefly, 5 × 10^4 cells were plated overnight on six-well plates (Corning Glassworks, Corning, NY). Cells were washed with phosphate-buffered saline with 1% bovine serum albumin (PBS/BSA) and detached after 10-min incubation with 2 mM EDTA in PBS. Cells were incubated with either anti-P-gp monoclonal antibody F4 or a matched mouse isotype control for 30 min at 4°C, and centrifuged at 20,000g. The supernatant was removed and fluorescence intensity of doxorubicin was measured with a fluorescence spectrophotometer F-4500 (Hitachi Instruments, San Jose, CA); excitation was set at 482 nm and emission at 560 nm.

**Cytotoxic Drug Sensitivity Assay.** LLC-PK1 control and MDR1 recombinant cells were plated at a density of 1 × 10^6 cells/well in a 96-well plate (Corning Glassworks) in complete media and allowed to attach for 4 h at 37°C. Varying concentrations of doxorubicin, vinblastine (Bedford Laboratories, Bedford, OH), and vincristine (Faulding, Paramus, NJ) were added to the cells in quadruplicate. Cells were incubated with the cytotoxic drugs for 3 days at 37°C. On the second day of the incubation, 1 μCi of [3H]thymidine (specific activity 81.1 Ci/mmol) was added to each well and incubated for 18 h. Cells were harvested with 5% trichloroacetic acid, and [3H]radioactivity was measured with a 1600 TR liquid scintillation analyzer (Canberra Industries, Zellik, Belgium). Cytotoxicity was measured as the effective concentration necessary for 50% cell death (EC_{50}) for each drug; EC_{50} values were estimated using a sigmoid E_{max} model on WinNonlin software (Pharsight, Mountain View, CA).

**Transepithelial Transport Assay.** LLC-PK1 control and recombinant MDR1 cells were plated at a density of 1 × 10^5 cells/well on permeable supports (Transwell; 3.0-μm pore size; Corning) and grown for 4 days; media were refreshed after 2 days in culture. Fresh media were added to the cells 1 h before the initiation of the experiment, and transepithelial electrical resistance values were measured with a Millicell ERS (Millipore, Billerica, MA). For transport of R123 across the epithelial cells, 1 μM R123 in Opti-MEM (Invitrogen) was added to either the apical or basolateral compartments with fresh Opti-MEM medium added to the opposite side. Inhibition was performed at 1 μM GF120918. Aliquots of 50 μl were taken from apical and basolateral compartments at 1, 2, 3, and 4 h. Fluorescence intensity of R123 was measured with a Gemini XS microplate spectrofluorometer (Molecular Devices, Sunnyvale, CA) with SoftMax Pro software (Molecular Devices); excitation was set at 488 nm and emission at 525 nm. Apparent permeability (P_{app}) was calculated in the apical-to-basolateral direction (P_{app, A→B}) and in the...
differences between two sets of data. 

**Statistical Analysis.** Student’s two-sided t test was used to evaluate differences between two sets of data. P values <0.05 were considered statistically significant.

**Results**

**Generation of Cells Expressing Recombinant MDR1**

MDR1wt or MDR11199. We have successfully constructed MDR1 vectors for mammalian cell expression containing either the wild-type or variant allele at nucleotide position 1199. The final plasmid constructs were verified by directly sequencing the MDR1 insert (data not shown). The introduction of the 1199A polymorphism from a wild-type plasmid was based on a restriction enzyme approach and is described in Fig. 1 (additional details are described under Materials and Methods). These plasmids, referred to as MDR1wt and MDR11199, were used for generating stable recombinant cells for functional studies.

We chose LLC-PK1 cells as the host to isolate stable epithelial cells for continuous expression of MDR1. These cells differentiate to form polarized monolayers for transepithelial efflux studies. As mammalian cells, LLC-PK1 should provide proper post-translational modification, which has been demonstrated to be important in targeting of P-gp to the plasma membrane and P-gp function (Loo and Clarke, 1993a,b, 1994a,b). To further reduce the baseline levels of P-gp-like activity in these cells, we have selected and cloned a line of LLC-PK1 cells that were P-gp negative based on their retention of R123 and sensitivity to the cytotoxic P-gp substrate doxorubicin. P-gp-deficient LLC-PK1 cells were transfected with respective plasmids by electroporation, and systematically selected for high levels of P-gp expression. The recombinant cells expressing MDR1wt or MDR11199 in a stable and consistent manner were cloned and expanded for functional studies. Direct sequencing was performed to verify expression of the correct allele at nucleotide position 1199 (Fig. 2). Image deconvolution also confirmed that MDR1wt and MDR11199 cells express similar amounts of P-gp and that expression is predominantly on the apical membrane (Fig. 4).

**P-gp-Mediated R123 Efflux.** Flow cytometry was used to evaluate efflux transport activity of MDR1wt and MDR11199 recombinant cells by measuring intracellular accumulation of R123, a fluorescent P-gp substrate. Mean intracellular R123 fluorescence (given in arbitrary units) for MDR1wt and MDR11199 cells was 3.91 ± 0.11 and 18.56 ± 0.46 (p < 0.001), respectively, an approximate 4.75-fold higher accumulation of R123 in MDR11199 cells (Fig. 5A). To verify that the R123 efflux observed was due to P-gp activity, we next used a specific and potent P-gp inhibitor, GF120918, for R123 efflux assessment. Although there was differential efflux observed between cells expressing MDR1wt or MDR11199, R123 efflux activity was abolished in both types of cells, reversing cellular R123 accumulation similar to that of control LLC-PK1 cells (Fig. 5, B–D). Dose-dependent analysis of the ability of GF120918 to inhibit P-gp-mediated R123 efflux in MDR1wt and MDR11199 cells was completed, and IC50 values were estimated. Similar IC50 values for inhibition by GF120918 in MDR1wt and MDR11199 were recorded as 20.3 ± 10.31 and 28.6 ± 2.1 nM, respectively. These results suggest that recombinant cells expressing MDR1wt or MDR11199 proteins are both capable of mediating P-gp-specific R123 transport activity, but cells expressing MDR11199 are less efficient than those expressing MDR1wt.

**Sensitivity to Cytotoxic Agents.** To evaluate the impact of differential efficiency in efflux activity of cells expressing MDR1wt or MDR11199, we used three cytotoxic chemotherapeutic agents that have been shown to exhibit P-gp-dependent drug resistance. Dose-response analysis was performed with P-gp substrates doxorubicin, vincristine, and vinblastine; these compounds were chosen because they have been shown to exhibit differential sensitivities across various MDR1 mutant cells generated by site-directed mutagenesis (Loo and Clarke, 1993a,b, 1994a,b). Drug sensitivities were estimated by EC50 values for each cytotoxic drug in LLC-PK1 control and MDR1wt and MDR11199 cells and are presented...
suggest that expression of both G1199A polymorphism. Significant progress has been made in the discovery of MDR1 polymorphisms and the assessment of allelic frequencies. The search for key genetic determinants, including MDR1 genetic polymorphisms, which alter the disposition of drugs that are substrates or inhibitors of P-gp in individuals, has just begun. Toward this end, we have developed stable recombinant LLC-PK1 cells expressing either MDR1wt or MDR11199 and evaluated the significance of the G1199A polymorphism.

Alterations in the efflux transport and chemoresistance of P-gp due to the G1199A transition have been observed in our recombinant expression system. Variations in efflux were...
demonstrated with the fluorescence substrate R123; cells expressing MDR11199 displayed decreased efflux of R123 compared with MDR1wt. Regardless of the efficiency of efflux, cells expressing either MDR1wt or MDR11199 were both inhibited to the same degree by a P-gp-specific inhibitor, GF120918. This indicates that the observed differences were due to the influence of an MDR1 polymorphism on P-gp function. Variation in chemoresistance due to G1199A polymorphism was also observed (Table 1). Cells expressing MDR11199 seem to be more resistant to vinblastine and vincristine than cells expressing MDR1wt; however, MDR1wt and MDR11199 recombinant cells displayed similar resistance to doxorubicin. Site-directed mutagenesis studies have also shown that modifications in the nucleotide sequence of MDR1 alter substrate efflux of some anticancer agents but do not cause a change in efflux of others (Loo and Clarke, 1993a,b, 1994a,b). Multiple binding domains in the drug-binding pocket of P-gp have been proposed and may account for the drug-specific alteration in P-gp efflux due to genetic polymorphisms (Martin et al., 2000; Loo et al., 2003). The observed differential sensitivity to cytotoxic agents due to G1199A may be important in modulating the efficacy of chemotherapy. The reported results could be used to provide alternate choices of drugs to overcome chemoresistance in some cancer patients. The use of LLC-PK1 epithelial cells as host cells allows for assessment of the role of MDR1 genetic variation in P-gp-dependent directional transcellular efflux. Permeability and directional efflux transport studies with the epithelial cells demonstrated that MDR11199 recombinant cells exhibited a lower PappB/PappA ratio for R123 (Table 2). Changes in drug permeability may impact absorption, bioavailability, and distribution into target tissues, including the central nervous system.

The variations in P-gp activity due to G1199A were found in our recombinant expression system are not comparable with those reported on MDR11199 expressed in HeLa cervical adenocarcinoma cells using a vaccinia virus-based transient expression system (Kimchi-Sarfaty et al., 2002). However,
the HeLa cell system cannot evaluate transcellular permeability, which is an especially important component of P-gp activity, particularly in intestinal absorption and blood-brain barrier penetration. In this transient expression system, HeLa cells expressing MDR11199 did not exhibit significant differences in efflux of bodipy-FL-verapamil, bodipy-FL-vinblastine, calcein-AM, bodipy-FL-prazosin, bisantrene, and bodipy-FL-forskolin, and daunorubicin (Kimchi-Sarfaty et al., 2002). However, the fluorescent bodipy modification on the P-gp substrates in this study may influence the ability to detect functional variability due to MDR1 polymorphisms. It is also possible that expression of some vaccinia viral elements and cytolytic viral proteins in these transient expressed HeLa cells contribute to the discordance. Our system allows for long-term expression of P-gp variants without the complication of viral elements. Whether coexpression of viral components influences P-gp function is not known and may require further investigation.

A considerable amount of contradictory reports exist in the literature as to the influence of MDR1 polymorphisms (primarily G2677T and C3435T) on P-gp expression and function at the cellular level. In vitro retroviral vectors containing MDR1 variants at 2677, designed to express Ala893 or Ser893 in NIH-3T3 mouse embryonic cells, suggested that Ser893-expressing cells exhibited decreased digoxin accumulation compared with Ala893 cells, indicating enhanced P-gp efflux (Kim et al., 2001). On the other hand, a vaccinia virus-based transient expression system of several MDR1 polymorphisms (A61G, T307C, G1199A, G2677T, and G2995A) in HeLa cells showed no difference in P-gp expression and efflux of various drugs (Kimchi-Sarfaty et al., 2002). Similarly, expression of different combinations of variations at 2677 and 3435 in LLC-PK1 cells (2677G/3435C, 2677G/3435T, 2677A/3435C, 2677A/3435T, 2677T/3435C, and 2677T/3435T) were shown to have no apparent difference in transcellular transport and accumulation of verapamil,
digestin, vinblastine, and cyclosporine (Morita et al., 2003). At present, the data in the literature provide no consistent in-
formation on the influence of MDR1 polymorphisms in vitro.
There is great potential for an in vitro transport system, based on recombinant MDR1 variants stably expressed in
epithelial cells, that will allow for studying the impact of MDR1 SNPs and attempt to sort out some of the controversy
in the literature.
In summary, we have developed a stable expression system for MDR1 in epithelial cells and demonstrated that G1199A
causes functional variance in efflux transport, drug resis-
tance, and transepithelial transport and permeability. De-
convolution microscopy studies have shown that stable recombinant cells express P-gp in the correct cellular location
and orientation, which has been confirmed by transepithelial analyses for MDR1<sub>wt</sub> and MDR1<sub>1199</sub> recombinant cells.
Elu-
cidating the significance of the G1199A polymorphism is the
first step. Our system may have substantial impact in pro-
viding a method to consistently express other MDR1 SNPs
and haplotypes to characterize the functional significance
of MDR1 polymorphisms.

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ment in HIV-1-infected individuals with allelic variants of the multidrug resis-

### Table 2

<table>
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<tr>
<th>LLCPK1 Cells</th>
<th>P&lt;sub&gt;appB&lt;/sub&gt; - P&lt;sub&gt;appA&lt;/sub&gt;</th>
<th>R123</th>
<th>R123 + GF120918</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0.90 ± 0.20</td>
<td>1.01 ± 0.33</td>
<td></td>
</tr>
<tr>
<td>MDR1&lt;sub&gt;wt&lt;/sub&gt;</td>
<td>3.54 ± 0.94&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96 ± 0.22</td>
<td></td>
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<tr>
<td>MDR1&lt;sub&gt;1199&lt;/sub&gt;</td>
<td>2.02 ± 0.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.56 ± 0.03</td>
<td></td>
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</table>

<sup>a</sup> Comparison of MDR1<sub>1199</sub> cells with MDR1<sub>wt</sub> cells; *p < 0.05.
<sup>b</sup> Comparison of MDR1<sub>wt</sub> and MDR1<sub>1199</sub> cells with control cells; †p < 0.01.

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TABLE 2

Apparent permeability ratios for rhodamine-123 transport in LLCPK1 control and recombinant MDR1 cells.


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