KINETIC CHARACTERIZATION OF P-GLYCOPROTEIN-MEDIATED EFFLUX OF RHODAMINE 6G IN THE INTACT RABBIT LUNG

David L. Roerig, Said H. Audi, and Susan B. Ahlf

Research Service, V.A. Medical Center, Milwaukee, Wisconsin (D.L.R., S.B.A.); Department of Anesthesiology (D.L.R.) and Department of Pulmonary and Critical Care Medicine (S.H.A.), Medical College of Wisconsin, Milwaukee, Wisconsin; and Department of Biomedical Engineering, Marquette University, Milwaukee, Wisconsin (S.H.A.)

Received March 30, 2004; accepted June 9, 2004

ABSTRACT:
P-Glycoprotein (P-gp) is an ATP-dependent drug efflux transporter involved in multidrug resistance and drug disposition in many organ systems. A majority of P-gp substrates are lipophilic amine drugs which also exhibit rapid extensive accumulation in lung tissue. P-gp is expressed in lung tissue, and the very nature of this drug efflux mechanism suggests a moderating role in pulmonary drug disposition. Little is known about P-gp-mediated efflux out of lung tissue or its kinetic characteristics as they may relate to the impact of P-gp on pulmonary drug accumulation. The present study develops an experimental and kinetic model to characterize the kinetics of P-gp-mediated efflux of rhodamine 6G dye (R6G) out of the intact rabbit lung. The perfusate concentration of R6G with time during recirculation through an isolated perfused rabbit lung was measured, and 66.6 ± 2.6% (S.E.) of the perfusate R6G was taken up by the lung. In the presence of P-gp inhibitors, R6G uptake increased significantly to 87.5 ± 1.1% (P < 0.002), indicating a functional pulmonary P-gp efflux transporter. Fractional lung accumulation of R6G increased with increasing R6G perfusate concentration, a result consistent with saturation of an efflux transporter. A parsimonious three-compartment kinetic model of R6G pulmonary disposition was used to interpret data sets from experiments with different perfusion variables and to estimate parameters descriptive of the dominant kinetic processes involved in R6G pulmonary accumulation. The estimated value of the kinetic parameter, k_{pgp}, rate constant for P-gp-mediated R6G efflux, indicates that this transporter plays a significant role in moderating R6G pulmonary disposition.

P-Glycoprotein (P-gp) is a 170,000-Da transmembrane phosphoglycoprotein that was first correlated with decreased drug accumulation and increased drug resistance in certain cell lines (Ford and Hait, 1990; Gottesman and Pastan, 1993; Ambudkar et al., 1999). P-gp is a member of the ATP-binding cassette transporter family and is encoded by the MDR1 and MDR2 genes in humans and the mdr1a, mdr1b, and mdr2 genes in rodents (Thiebaut et al., 1987; Borst et al., 1993). Overexpression of P-gp is recognized as an important mechanism in the development of multidrug resistance in cancer chemotherapy. P-gp is also present in normal tissue (Thiebaut et al., 1987; Endicott and Ling, 1989; Sharom, 1997). In humans, P-gp is located in the plasma membrane in specific cell types of different organs including intestine, kidney, and lung and in capillary endothelial cells of the brain wherein roles for P-gp in drug uptake, metabolism, excretion, and limiting central nervous system concentrations, respectively, have been reported (Endicott and Ling, 1989; Ford and Hait, 1990; Ambudkar et al., 1999). In brain, P-gp appears to be a functional aspect of the blood-brain barrier (Cordon-Cardo et al., 1989; Schinkel et al., 1996; Regina et al., 1998). Using various methods, P-gp expression has been reported in other organs including lung (Jette et al., 1996; Demeule et al., 1999). Brady et al. (2002) found that mdr1b mRNA expression in the lung was second only to the gastrointestinal tract. Other studies have located P-gp in bronchial and alveolar epithelial cells and in pulmonary and bronchial capillary endothelial cells (Lechapt-Zalcman et al., 1997; Demeule et al., 2001; Campbell et al., 2003).

P-Glycoprotein has been referred to as an atypical ATP-dependent transporter that is highly promiscuous based on the hundreds of compounds that have been identified as substrates (Sharom, 1997). The physical chemical properties of P-gp substrates are diverse and most commonly include some degree of lipophilicity and a positive charge, and/or an amine nitrogen (Frezzard et al., 2001). Seelig (1998) reviewed 100 compounds that interact with P-gp, and more than 60 were basic lipophilic amines, a property shared with compounds that readily accumulate in lung tissue. The lung is well known for its extensive accumulation of lipophilic amines, and this similarity to the substrate specificity of P-gp plus the very nature of this drug efflux transporter strongly suggest that P-gp-mediated drug efflux could play a moderating role in the pulmonary accumulation of amine drugs (Junod, 1976; Wilson et al., 1979; Roerig et al., 1983; 1984; Bend et al., 1985). However, being a substrate for P-gp does not ensure that
this efflux transporter will significantly moderate drug disposition in lung tissue. For an intact organ, tissue accumulation is dependent on multiple, and often competing, vascular and intracellular dispositional processes. These include rate of delivery to the organ (blood flow), diffusion into or out of specific cell types, interactions such as binding to intracellular sites, or accumulation in subcellular organelles. An additional factor is saturation of one or more of these kinetic processes, which could reduce the impact of the saturable process on overall tissue accumulation of the drug.

This work is aimed at developing a kinetic model of pulmonary drug disposition to identify the contribution of the different kinetic processes, including P-gp-mediated drug efflux, in the lung tissue accumulation of lipophilic amines. Because of its ease of ex vivo perfusion, the ability to manipulate perfusion variables, and expression of P-gp in normal lung tissue, the lung may also serve as an organ model in general. For this study, we selected the lipophilic amine dye, rhodamine 6G (R6G) as the test substrate based on its reported uptake in cells (Loetchutinat et al., 2003).

**Materials and Methods**

**Chemicals and Reagents.** R6G was obtained from Sigma-Aldrich (St. Louis, MO). Sodium pentobarbital (50 mg/ml), heparin sodium (1000 units/ml), and verapamil HCl were obtained from Abbott Laboratories (Abbott Park, IL), Elkins-Sinn Inc. (Cherry Hill, NJ), and Knoll Pharmaceutical Co. (Mt. Olive, NJ) respectively. Bovine serum albumin (standard powder) was purchased from Se-rologicals Corp. (Norcross, GA). The P-glycoprotein inhibitor, N-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isooquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carbazide (GF120918), was generously supplied by GlaxoSmithKline (Uxbridge, Middlesex, UK) and dissolved in dimethyl sulfoxide (1 mg/ml). All other chemicals were of reagent grade.

**Animals.** All studies were carried out with New Zealand White rabbits of either gender with a mean ± S.E. weight of 2.65 ± 0.2 kg. The rabbits were housed one per cage, with free access to food and water, and maintained on a 12-h light/dark cycle. All rabbits were purchased from New Franken Research Rabbits (New Franken, WI). All animal care and treatment procedures were approved by the V.A. Medical Center Research Institutional Animal Care and Use Committee before initiating these studies.

**Isolated Lung Perfusion System.** The isolated perfused rabbit lung (IPL) system was similar to that previously described (Roerig et al., 1992; Audi et al., 1995, 1996). Briefly, rabbits were anesthetized with sodium pentobarbital (25–40 mg/kg, i.v., ear vein), and the carotid artery was cannulated. The rabbits were heparinized (1000 units/kg) and exsanguinated via the carotid cannula. The chest was opened and the pulmonary artery, pulmonary vein, and trachea were cannulated. The lung was removed from the chest and suspended in the perfusion apparatus by the cannulas, and the perfusion system was primed with a perfusate consisting of a physiological salt solution containing 4.5% bovine serum albumin. Perfusion was initiated pumped through the IPL at 200 ml/min (Master Flex roller pump; Cole-Parmer Instrument Co., Vernon Hills, IL), and the uniformity of branching after the residual blood was washed out of the lung was used to estimate the extent of perfusion of the IPL. Only lungs greater than 90% perfused were used for these studies. The venous outflow was then directed to the reservoir supplying the pump. Additional perfusate was added to the reservoir to give a recirculating perfusate volume of 134 ± 1 ml. The pulmonary artery pressure, referenced to the level of the left atrium, was 8.1 ± 0.3 cm of H2O at end expiration, and IPLs that exhibited an arterial perfusion pressure greater than 10 cm of H2O were not studied. The perfused lung was maintained at 37°C. The lungs were continuously ventilated (10 breaths/min) with a gas mixture containing 15% O2, 5% CO2, balance N2, with end-inspiratory and –expiratory airway pressures of 7.7 ± 0.1 and 2.1 ± 0.1 cm of H2O, respectively. At the end of each experiment the lungs were weighed and lyophilized to a constant weight. The wet/dry weight ratio of all lungs was 6.11 ± 0.06.

**Experimental Protocols.** The uptake of R6G with time was determined by adding known amounts of R6G to the recirculating reservoir and then removing 1.7 ml of perfusate samples from the reservoir at fixed time intervals out to 120 min, depending on the particular experiment. The sampling interval after R6G addition to the reservoir was every 5 min for the first 30 min, every 10 min between 30 and 60 min, and every 15 min thereafter. Depending on the experiment, the P-gp inhibitor, GF120918, was added to the perfusate reservoir 5 min before the R6G or at various times after R6G addition.

A standard concentration curve for R6G was constructed using perfusate that had recirculated through the lung before R6G addition and a portion of the stock R6G solution that was added to the recirculating reservoir. All samples and standards were centrifuged to remove trace red blood cells, and the absorbance at 540 nm was used to determine the R6G perfusate concentration. The amount of R6G taken up by the lung was determined by its disappearance from the recirculating reservoir corrected for the amount of R6G removed from the perfusion system during sampling. Each lung was used only once, and the number (n) of individual rabbit lungs used in the different experiments is given in the figure legends.

**Results**

**Uptake of R6G in the Lung.** Figure 1 shows the nanomoles of R6G in the isolated perfused rabbit lung with time after 2.5 μM R6G was added to the recirculating reservoir in control lungs (Fig. 1, open circles) and in lungs to which the P-gp efflux inhibitor verapamil (Fig. 1, closed triangles; Ford and Hait, 1990) or GF120918 (Fig. 1, closed circles; Hyafil et al., 1993) were added to the perfusate reservoir 5 min before addition and recirculation of the R6G. With either inhibitor, the R6G accumulation increased. At all time points, R6G accumulation was significantly greater in the presence of GF120918 (P < 0.002). At 90 min after the start of R6G recirculation, 87.5 ± 1.1% (S.E.) of the R6G had accumulated in GF120918-treated lungs compared with 66.6 ± 2.6% for control lungs.

Figure 2 depicts a family of curves showing the increase in the total nanomoles of R6G in the rat lungs versus time with initial perfusate R6G concentrations ranging from 1.25 μM to 12.6 μM. This experiment provides one of the discriminating data sets necessary to estimate values of the kinetic model parameters (see “Kinetic Model and Data Analysis”). This experiment also provides insight into possible saturation of dispositional processes involved in R6G lung accumulation. For example, in the absence of any saturation, the fraction of
the initial amount of R6G in the perfusate in the lung at the end of the perfusion should be constant over the R6G perfusate concentration range studied. However, as shown in Fig. 3, the fraction of total R6G in the lung at 90 min increased as a function of total R6G initially present in the reservoir.

The effect of GF120918 concentration on R6G accumulation in the lung was also determined. Lungs were perfused for 5 min with perfusate concentrations of GF120918 of 0, 2, 10, 20, and 70 μM before recirculation of 2.5 μM R6G. Accumulation of R6G in the lung was then determined at each inhibitor concentration and is shown in Fig. 4. The accumulation of R6G in the lung increased with increasing GF120918 perfusate concentration. This experiment also provides a data set necessary to estimate values of the kinetic model parameters as explained under “Kinetic Model and Data Analysis,” described below. It is apparent in Fig. 4 that inhibition of P-gp is approaching a maximum, and the 20 μM GF120918 concentration (Fig. 4, open triangles) used to inhibit P-gp-mediated R6G efflux out of the lung in Figs. 1 and 5 results in near maximal P-gp inhibition.

In an additional study, 20 μM GF120918 was added to the recirculating perfusate at various times after the start of R6G recirculation through the lung. Figure 5 shows the increase in the total R6G in the lung versus time with GF120918 added at the times indicated by the arrows. Addition of the P-gp inhibitor caused a sudden increase in R6G accumulation that was greater the earlier the GF120918 was added to the recirculating perfusate.

**Kinetic Model and Data Analysis.** To evaluate the effect of P-gp and other cellular processes on R6G accumulation in the lung (Figs. 2–5), we developed a parsimonious kinetic model for the pulmonary disposition of R6G. The model (Fig. 6) consists of three compartments, a vascular compartment and two extravascular compartments. The vascular compartment (compartment 1) represents the lung vascular region, reservoir, and tubing. The two extravascular compartments (compartments 2 and 3) represent distribution of R6G within the lung tissue. The model allows for R6G diffusion between compartments 1 and 2 with the rate of diffusion represented by the permeability-surface area product, P5 (ml/min); P-gp-mediated efflux of R6G from compartment 2 into compartment 1, described by a Michaelis-Menten-type process with an apparent maximum efflux rate $V_{\text{max}21}$ (nmol/min) and a Michaelis constant $K_{m21}$ (nmol); accumulation of R6G in compartment 3, also described by a Michaelis-Menten-type process with an apparent maximum uptake rate $V_{\text{max}23}$
(nmol/min) and a Michaelis constant $K_{m23}$ ($\mu$M); and a passive intercompartmental transfer from compartment 3 to compartment 2, described by the first order rate constant $k_{32}$ (min$^{-1}$). Compartment 3 could represent intracellular binding sites or sequestration in organelles. The extensive pulmonary uptake of R6G suggests that this is a high-capacity, high-affinity intracellular pool for R6G. Thus, in eqs. 1 to 3, below, $V_{m23}$ and $K_{m23}$ are replaced by the first order rate constant $k_{32} = V_{m23}/(K_{m23}V_2)$ (min$^{-1}$). The inhibitor GF120918 is assumed not only to inhibit P-gp-mediated R6G efflux with an inhibition constant $K_i$ ($\mu$M), but also to inhibit R6G accumulation in compartment 3 with an inhibition constant $K_{i2}$ ($\mu$M). The kinetic model makes no assumption of the anatomical location of the P-gp protein but does assume that P-gp transports R6G from the lung tissue into the vascular space.

The temporal variations in the concentrations of the various species in the three compartments are described by eqs. 1 to 3.

\[
\frac{d[C_1(t)]}{dt} = \frac{C_2(t)}{V_2} + \frac{V_{max21}}{K_{m21}V_2(1 + \frac{[i]}{K_i}) + C_2(t)} - \frac{PS[C_1(t)]}{V_1}
\]  

\[
\frac{dC_2(t)}{dt} = PS[C_1(t)] + k_{32}C_2(t) - C_2(t)\left(\frac{PS}{V_2} + \frac{V_{max21}}{K_{m23}V_2(1 + \frac{[i]}{K_i}) + C_2(t)} + \frac{k_{33}}{V_2}\right)
\]  

\[
\frac{dC_3(t)}{dt} = \frac{k_{32}C_2(t)}{V_2} - k_{i2}C_3(t)
\]

where $[C_1(t)]$ is the vascular (perfusate) concentration ($\mu$M) of R6G; $C_2(t)$ and $C_3(t)$ are the amounts (nmol) of R6G in compartments 2 and 3, respectively; $[i]$ is the concentration ($\mu$M) of the inhibitor GF120918 in the extravascular region; $\lambda$ is the inhibitor’s extravascular-to-vascular partition coefficient; $k_{32}$ (min$^{-1}$) is an approximation to the $V_{max21}/(K_{m23}V_2)$ ratio, and $V_1$ and $V_2$ (ml) are the volumes of compartments 1 and 2, respectively.

The identifiable model parameters are the following parameter groups: PS (ml/min); $k_{32} = PS/V_2$ (min$^{-1}$), a measure of the rate constant for diffusion-mediated lung efflux of R6G; $V_{max21}$ (nmol/min), measure of the affinity of P-gp for R6G; $V_{max23}$ (nmol/min), measure of the capacity of P-gp for R6G; $k_{33}$ and $k_{i3}$ (min$^{-1}$), first order rate constants for the transfer of R6G in and out of the subcellular pool (compartment 3), respectively; and $K_i/\lambda$ and $K_{i3}/\lambda$, respective measures of the inhibition constants for P-gp-mediated R6G efflux and R6G accumulation in compartment 3. The volume of compartment 1, $V_1$, was set at 134 ml.

The above model parameters are not estimable from a single set of data or experimental condition. In other words, the information content of a single set of data or experimental condition does not provide sufficiently discriminating information about all dominant processes to separately estimate all the model parameters. Thus, the approach that we have taken to estimate values for the kinetic model parameters was to carry out simultaneous nonlinear regression fits of the solution of eqs. 1 to 3 to all the mean data in Figs. 2 and 4 obtained by varying the perfusion conditions as indicated above. The model solution was corrected for the change in reservoir volume due to sampling. The estimated values of the model parameters are given in Table 1.
The lung is the increased fractional accumulation of R6G with increased efflux transporter P-gp in isolated perfused lungs from normal rabbits. This conclusion is based on the increased accumulation of the rhodamine dye R6G after the addition of the P-gp inhibitor GF120918 in the perfusion medium. The effect on the accumulation of R6G was also observed when the inhibitor was added at different times during the recirculation period.

To examine the contribution of P-gp-mediated efflux to the pulmonary uptake of R6G, we compared the estimated values of the kinetic parameters $k_{\text{up}}$ for R6G uptake ($V_{\text{max}1}/K_{\text{m1}}$, $k_{\text{out}} = PS/V_s$, $k_{\text{diff}}$) and $k_{\text{out}}$ for R6G efflux ($V_{\text{max}2}/K_{\text{m2}}$, $k_{\text{diff}} = PS/V_s$, $k_{\text{out}} = PS/V_s$) with the rate constants ($\text{min}^{-1}$) for the competing dispositional processes, namely P-gp-mediated efflux, non-P-gp-mediated efflux, diffusion into the lung, and intracellular transfer into the subcellular pool (see Table 1).

A Fortran-based computer program (Fortran PowerStation, Version 4.0) was developed for evaluating eqs. 1 to 3 (kinetic model) and for estimating the model parameters descriptive of the various processes (Audi et al., 1998). As described above, for the appropriate initial conditions, the model equations were solved numerically (Gear’s method) using International Mathematics and Statistics Library (IMSL) subroutine DBCLSF (IMSL Math/Library). The optimization for fitting the model to the data and parameter estimation was carried out using IMSL subroutine DBCLS, which finds the best nonlinear least-squares solution using a modified Levenberg-Marquardt algorithm with finite difference Jacobian. The 95% asymptotic parameter confidence intervals (Table 1) were calculated from the sensitivity matrix as previously described (Audi et al., 1998).

### Discussion

This study provides evidence for the expression of the multidrug efflux transporter P-gp in isolated perfused lungs from normal rabbits. This conclusion is based on the increased accumulation of the rhodamine dye, R6G, in the presence of verapamil or GF120918 (Figs. 1, 4 and 5), two known P-gp inhibitors (Ford and Hail, 1990; Hyafil et al., 1993). Also, consistent with P-gp-mediated transport of R6G out of the lung is the increased fractional accumulation of R6G with increasing perfusate R6G (Fig. 3). This was interpreted as saturation where the P-gp-mediated efflux rate of R6G approaches a constant, $V_{\text{max}2}$, resulting in a proportionately decreased effect of P-gp on R6G disposition at the higher R6G perfusate concentrations.

In normal lung tissue, P-gp is expressed in both airway epithelial cells and lung endothelial cells (Lechapt-Zalman et al., 1997; De Meule et al., 2001; Campbell et al., 2003). Campbell et al. (2003) suggested a protective role of epithelial P-gp as a barrier to xenobiotic transfer from alveoli to pulmonary interstitium and blood. We have focused on the role of P-gp in pulmonary drug accumulation from the circulation, where a protective role of P-gp from blood-borne xenobiotics is of great potential importance, as well as an even larger potential role in limiting pulmonary accumulation of a large number of systemic therapeutic agents where the lung is the target organ. The accessibility to this transporter from the vascular space and the reported presence of P-gp in pulmonary endothelial cells suggests that endothelial P-gp moderates pulmonary R6G accumulation (Demeule et al., 2001). However, pulmonary epithelial P-gp must also be considered. Epithelial P-gp would transport and retain R6G in the airspace rather than transport it out of the lung back into the circulation. Inhibition of epithelial P-gp would therefore decrease net lung R6G accumulation. This is the exact opposite of our results, suggesting that epithelial P-gp contributes little to the observed kinetics of pulmonary R6G accumulation from the vascular space.

The promiscuous nature of P-gp substrate specificity parallels the affinity of lipophilic amine drugs for lung tissue. Depending on the drug, accumulation of lipophilic amines in the intact lung results from both rapidly and slowly equilibrating interactions with lung tissue (Audi et al., 1995, 1996, 1998; Roerig et al., 1999). To study this differential amine interaction with macromolecules and/or partitioning among subcellular organelles, we developed kinetic models of pulmonary drug disposition to identify and characterize kinetic parameters descriptive of different drug-tissue interactions in the vascular and extravascular space (Audi et al., 1998, 2002; Roerig et al., 1999). We used a similar approach to develop a kinetic model for pulmonary R6G accumulation in the intact lung that includes kinetic parameters descriptive of the role of P-gp-mediated efflux and other drug-dispositional mechanisms inferred by the data in Figs. 1 through 4. Based on Fig. 3, P-gp-mediated efflux of R6G is saturable and is assumed to follow Michaelis-Menten kinetics as proposed by others (Weiss and Kang, 2002). The extensive net accumulation of R6G over the 90 min of recirculation (Figs. 1, 2, 4, and 5) indicates that at steady state, distribution is far in favor of the lung, suggesting a large apparent lung tissue volume of distribution. Since R6G is known and marketed as a mitochondrion-selective dye, compartment 3 (Fig. 6) represents, at least in part, sequestration in a subcellular organelle (Haugland, 2002). Other slowly equilibrating R6G-lung tissue interactions may also be involved but cannot be separately identified with the present data. Weiss and Kang (2002) developed a similar compartmental model to describe the role of P-gp in idarubicin disposition in the isolated perfused heart and proposed a carrier-mediated transport process from compartment 2 to 3. Again, this cannot be separately identified from the data presented here. The model (Fig. 6) developed from these considerations was simultaneously fit to the data in Figs. 2 and 4 and, as can be seen, resulted in a reasonably good fit (solid line) to the experimental data points. To explain (fit) all the data in Figs. 2 and 4, it was necessary to include an additional dispositional kinetic parameter, $K_{\text{m}}$, that describes inhibition of R6G accumulation in compartment 3 by the P-gp inhibitor, GF120918. The model was further tested by using the model parameter estimates obtained from fitting the data in Figs. 2 and 4 to predict the shape of the outflow curves in Fig. 5 where the P-gp inhibitor GF120918 was added at various times after the start of R6G recirculation. As can be seen from the solid lines in Fig. 5, the model faithfully predicts the increase in R6G accumulation that occurs after GF120918 addition at different times during the recirculation period.

The kinetic model parameters obtained from the solid lines are shown in Table 1. The effect of P-gp-mediated R6G influx relative to the other dispositional kinetic processes can be examined by comparing the rate constants ($\text{min}^{-1}$) for P-gp-mediated efflux ($k_{\text{up}}$), non-P-gp-mediated efflux ($k_{\text{out}}$), diffusion into the lung ($k_{\text{diff}}$), and sequestration into compartment 3 ($k_{32}$) using the model parameters values in Table 1. If P-gp were fully inhibited or not present, sequestration in compartment 3 ($k_{32}$ = 0.22 min$^{-1}$) would dominate over back-diffusion ($k_{\text{out}}$ = 0.011 min$^{-1}$), resulting in near unidirectional influx and sequestration in the lung. This is supported by the fact that after 90 min of R6G recirculation in the presence of an inhibitor (Fig. 1), 87.5 ± 1.1% (S.E.)
of the R6G was in the lung. The value of $k_{\text{ref}}$ of 1.44 min$^{-1}$ is 2 orders of magnitude greater than that of $k_{\text{net}}$, suggesting that P-gp-mediated efflux is by far the dominant efflux mechanism. In addition, $k_{\text{ref}}$ is 15 times greater than $k_{\text{net}} = 0.093$ min$^{-1}$ and 6 times the rate constant for sequestration ($k_{\text{seq}} = 0.22$ min$^{-1}$), resulting in P-gp-mediated efflux significantly moderating the amount of R6G available for disposition into compartment 3 and the lung as a whole.

The inhibition of R6G accumulation into compartment 3 in the presence of GF120918 is interesting since it poses a mechanism that would offset the effect of P-gp inhibition. GF120918 is a lipophilic amine, and inhibition of sequestration of one lipophilic amine by another in the lung has been reported (Anderson et al., 1974; Wilson et al., 1979; Roerig et al., 1989). This mechanism may be of some importance since reversal or modulation therapy using inhibitors of P-gp has been proposed and studied as a method of reversing the development of multidrug resistance in cancer chemotherapy (Ford and Hait, 1990).

Others have proposed models to estimate kinetic parameters to provide insight into the role of P-gp-mediated drug efflux in drug disposition. Loechtutinat et al. (2003) estimated the rate constants, $k_r$ and $k_a$, for P-gp-mediated efflux and passive membrane permeability, respectively, of different rhodamine dyes in a human leukemia cell line. For R6G, the ratio of $k_r/k_a$ was about 29, indicating that P-gp also dominated the rate of R6G efflux out of the cell in their studies. Wielinga et al. (2000) also identified a kinetic parameter for passive permeation and a measure of P-gp-mediated efflux using four anthra-cyclines analogs in an MDR cell line. They concluded that passive permeation plays a substantial role in determining the drug resistance for these anthracyclines. Wielinga et al. (2000) also identified a rate constant, $k_p$, for passive transport between an inner compartment and a shell compartment of the cell. Based on their data, like our findings with R6G, the rate constant for transport into an intracellular sequenced compartment was greater than that of the passive permeation parameter. The results of both of the above studies demonstrated that the difference for passive membrane permeability between the different compounds studied did not parallel the differences in the derived rate constants for P-gp-mediated efflux, with high lipophilicity having more effect on passive efflux. Weiss and Kang (2002) developed a whole organ model for evaluating the role of P-gp in the uptake of idarubicin in the isolated perfused rat heart. Their three-compartment model contained kinetic parameters descriptive of different intracellular-lipid compartmental processes. They concluded that the rate of idarubicin uptake in the heart represents a balance between two opposing processes: active P-gp-mediated efflux and uptake into mitochondria. These studies, together with the present work, all serve to emphasize the importance of competing intracellular processes when defining the impact of P-gp-mediated drug efflux on overall tissue disposition.

In conclusion, these studies demonstrate a functional P-gp-mediated drug efflux transporter in the lung that significantly moderates the disposition of R6G between the vascular and extravascular space in the lung. Most importantly, the experimental protocol presented, together with the proposed kinetic model and appropriate experimental data sets, can provide estimates of kinetic parameters that are descriptive of the different dispositional mechanisms involved in pulmonary drug accumulation. Reliable estimates of all of these kinetic parameters will be essential in assessing the role of P-gp-mediated drug efflux in the net pulmonary accumulation of a large variety of therapeutic agents.

Reference


Address correspondence to: Dr. David L. Roerig, Research Service 151, V.A. Medical Center, 5000 W. National Ave., Milwaukee, WI 53295. E-mail: droerig@mcw.edu

Downloaded from dx.doi.org at ASPET Journals on June 22, 2017