P-Glycoprotein Inhibitors Enhance Saturable Uptake of Idarubicin in Rat Heart: Pharmacokinetic/Pharmacodynamic Modeling

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Received August 29, 2001; accepted November 2, 2001 This paper is available online at http://jpet.aspetjournals.org

ABSTRACT
Little is known about cardiac uptake kinetics of idarubicin, including a possible protective role of P-glycoprotein (Pgp)-mediated transport. This study therefore investigated uptake and negative inotropic action of idarubicin in the single-pass isolated perfused rat heart by using a pharmacokinetic/pharmacodynamic modeling approach. Idarubicin was administered as a 10-min constant infusion of 0.5 mg followed by a 70-min washout period in the absence and presence of the Pgp antagonists verapamil or amiodarone. Outflow concentration and left ventricular developed pressure were measured and the model parameters were estimated by simultaneous nonlinear regression. The results indicate the existence of a saturable, Michaelis-Menten type uptake process into the heart ($K_m = 3.06 \mu M, V_{max} = 46.0 \mu M/min$). Verapamil and amiodarone significantly enhanced the influx rate ($V_{max}$ increased 1.8-fold), suggesting that idarubicin is transported by Pgp directly out of the membrane before it gets into the cell. Verapamil and amiodarone attenuated the negative inotropic action of idarubicin, which was linked to the intracellular concentration of idarubicin.

Little attention has been paid to the kinetics of drug uptake into the myocardium, despite the clinical importance of these transport mechanisms for the efficacy and toxicity of cardioactive drugs. Thus, the clinical utility of the antineoplastic agent idarubicin is limited by a high incidence of severe and usually irreversible cardiac toxicity; however, the transport mechanism of idarubicin (and other anthracyclines) into the heart is still unclear. Previous studies in cell lines have shown contradicting results. In multiple drug resistance (MDR) cells, membrane permeability and hydrophobicity of anthracyclines were highly correlated (Wielinga et al., 2000), in accordance with the assumption that a highly lipophilic drug such as idarubicin would passively diffuse across the plasma membrane (Stein, 1997). However, saturable uptake of anthracyclines into cells also has been reported (Decorti et al., 1998; Sasaya et al., 1998). Furthermore, anthracyclines are well known substrates for P-glycoprotein (Pgp); however, as pointed out recently, there is relatively limited information on the functional role of Pgp and related transporters in the heart (Rodriguez et al., 1999). It has been suggested that Pgp acting as a drug efflux pump can decrease the cellular concentration of some drugs and may play an important role in the protection of the heart. An increased cardiac accumulation of vinblastine (van Asperen et al., 1999a) and doxorubicin (van Asperen et al., 1999b) has been reported in mice lacking mdr1a Pgp. The Pgp pump is inhibited by reversal agents for MDR; among these are verapamil and amiodarone (Stein, 1997). Indirect evidence for Pgp-mediated transport in the heart has been obtained from an enhancement of cardiac uptake of anthracyclines after combination with Pgp inhibitors (Colombo et al., 1996).

This study was designed to characterize the uptake process of idarubicin and to examine the effect of the Pgp antagonists verapamil and amiodarone in the single-pass perfused rat heart. To our knowledge, such a kinetic analysis of cardiac Pgp substrate transport has so far not been reported. The method is based on the measurement of venous outflow concentration-time profile and contractile response after a 10-min infusion of idarubicin into the inflow. However, the parameters that govern transport mechanisms are not directly observable and can only be obtained using a mathematical model that attempts to describe the disposition kinetics of the drug in the organ. Compartmental modeling quantified some basic features and provided evidence for Michaelis-Menten type uptake and Pgp-mediated influx “hindrance” of idarubicin. Furthermore, by pharmacokinetic/pharmacodynamic modeling cellular kinetics was linked with the time course of negative inotropic response of idarubicin. Thus, information on the functional role of compartments was ob-

ABBREVIATIONS: MDR, multiple drug resistance; Pgp, P-glycoprotein; IDA, idarubicin; LVDP, left ventricular developed pressure; SNLR, simultaneous nonlinear regression; CV, coefficient of variation of parameter estimate.

This work was partially supported by Deutsche Forschungsgemeinschaft (GRK 134/1-96).
tained. The goal of the study was to establish a model of cardiac uptake kinetics of the Pgp substrate idarubicin that describes quantitatively how changes in the transport processes will determine intracellular pharmacokinetics. However, our results are not only of interest for idarubicin as a lipophilic model compound and Pgp substrate but also they have clinical implications for a better understanding of anthracycline cardiotoxicity in cancer chemotherapy, especially regarding the potential interaction with modulators of MDR (Lambert et al., 1990; van Asperen et al., 1999a,b).

Materials and Methods

Perfused Rat Heart. All experiments used an isolated isovolumetrically contracting rat heart as previously described (Kang and Weiss, 2001). Male Sprague-Dawley rats, 300 to 350 g, were anesthetized with sodium pentobarbital (50 mg/kg i.p.). After injection of heparin (500 IU) into the tail vein, a cannula was bound into the trachea for ventilation. The chest was opened and an aortic cannula filled with perfusate was rapidly inserted into the aorta, and retrograde perfusion was started with an oxygenated Krebs-Henseleit buffer at 37°C. The pulmonary artery was incised to allow outflow of the perfusate. Coronary perfusion was initiated through a short cannula in the aortic root and maintained at a constant pressure of 60 mm Hg in a single-pass way by the Langendorff Technique. The flow was controlled by a roller pump. A latex balloon was placed in the left ventricle and connected to a pressure transducer line. The balloon was inflated with water to create a diastolic pressure of 5 to 6 mm Hg. After stabilization, the system was changed to constant flow condition maintaining a coronary flow of 9.5 ± 0.4 ml/min. The hearts were beating spontaneously at an average rate of 300 beats/min. Coronary perfusion pressure, left ventricular pressure, and heart rate were measured continuously and a physiological recording system (Hugo Sachs Elektronik, March, Germany) was used to monitor left ventricular systolic pressure, left ventricular end diastolic pressure, and maximum and minimum values of rate of left ventricular pressure development (LVdP/dt\text{max} and LVdP/dt\text{min}). Left ventricular developed pressure was calculated as LVDP = left ventricular systolic pressure − left ventricular end diastolic pressure.

Experimental Protocol. Hearts were perfused for 120 min in the absence (n = 5) or presence of the Pgp inhibitors verapamil (1 nM, n = 5) or amiodarone (1 μM, n = 5). These concentrations of verapamil and amiodarone were below the threshold values that lead to changes in the measured cardiovascular effects (much lower than the therapeutic concentrations of about 10 μM).

After a 20-min equilibration period, idarubicin was infused for 10 min with an infusion device into the perfusion tube close to the aortic cannula. Coronary venous outflow samples were collected every 30 s for 20 min, every 60 s for the next 10 min, and every 5 min for the next 50 min (total collection period 80 min). These samples were assayed for idarubicin by high-pressure liquid chromatography as previously described (Kang and Weiss, 2001).

Model Development and Data Analysis. A model must be constructed that not only describes the measured outflow concentration-time profiles and the time course of contractile response but also that is simple enough that it can be identified on the basis of the experimental data. We developed a minimal compartmental model that reflects the key aspects of myocardial distribution kinetics of idarubicin. The model development was an iterative process both with regard to the underlying data sets and the selected model structures. For the model structure shown in Fig. 2, the differential equations that describe changes in the amounts of idarubicin in compartments after infusion of idarubicin at the inflow side of the heart (single-pass mode) are given by eqs. 1 to 3:

\[ dx_i/dt = -(Q/V_1 + f_{V_{\text{max},1}}V_{\text{max},1}(K_{iV_{\text{max},1}} + x_1))x_1 + k_{21}x_2 + R \]

\[ dx_2/dt = (f_{V_{\text{max},1}}V_{\text{max},1}(K_{iV_{\text{max},1}} + x_1))x_1 \]

\[ -(k_{21} + k_a + V_{\text{max},2}(K_{iV_{\text{max},2}} + x_2))x_2 + k_{23}x_3 \]

\[ dx_3/dt = (V_{\text{max},2}(K_{iV_{\text{max},2}} + x_2))x_2 - k_{23}x_3 \]

Note that perfusate flow (Q) and drug input rate (R) as well as drug outflow are assumed to occur in compartment 1 (distribution volume V_1) representing the vascular space and rapidly equilibrating tissue regions with respect to the myocardial disposition of idarubicin. First order rate constants describing (passive) intercompartmental transport are denoted by k_j, and the active transport with Michaelis-Menten type kinetics is characterized by the apparent maximal transport rates V_{\text{max},ij} and the apparent Michaelis constants K_{iV_{\text{max},ij}}. The factor f_{V_{\text{max},1}} accounts for the effect of verapamil (f_{V_{\text{max},12,vera}}) or amiodarone (f_{V_{\text{max},12,amio}}) on the pharmacokinetics of idarubicin (f_{V_{\text{max},12,control}} = 1). Combined kinetic-dynamic modeling was performed linking the time course of idarubicin amount in the effect site compartment x\text{eff}(t) described by the following equation:

\[ dx\text{eff}/dt = (x_2 - x_\text{eff})v_{\text{rat}} \]

with the time course of its negative inotropic action of idarubicin, E(t). The response or transit time t_{\text{eff}} characterizes the disequilibrium between the functional effect site (x\text{eff}) and compartment 2 (Holford and Sheiner, 1981; D’Argenio and Schumitzky, 1997). The effect E(t) was defined as the decrease of LVDP as fraction of the baseline response LVDP(0):

\[ E(t) = -\text{LVDP}(0) - \text{LVDP}(t) \]

\[ \text{LVDP}(t) \]

The effect site concentration (amount) induces the effect E(t) by a sigmoid E_{\text{max}} model (Holford and Sheiner, 1981):

\[ E(t) = E_{\text{max}}(x_{\text{eff}}(t))^{N} \]

\[ \text{EX}_{\text{iso}} + x_{\text{eff}}(t) \]

where EX_{iso} is the effect site amount that corresponds to 50% of the maximum effect (E_{\text{max}}) and N is the Hill coefficient that determines the sigmoidicity of the curve. Note that in the above-mentioned empirical model t_{\text{eff}} also accounts for delays in the effectuation process (Paschoal et al., 1998).

Equations 1 to 4 were solved numerically and fitted to the data by using the ADAPT II software package (D’Argenio and Schumitzky, 1997) with the error model as follows:

\[ \tilde{C}(t) = C(t) + e_i \]

\[ \text{var}[e_i(t)] = (a_{\text{es}} + \sigma_{\text{es}}C(t))^{2} \]

where \( t \) denotes the measured concentration and \( \tilde{C}(t) = x_{\text{eff}}(t)\text{V}_{1} \) is the model prediction.

The crucial associated question is concerned with the uncertainties in the estimated parameter values because model identifiability is a central problem in using models with saturable transport processes (nonlinear systems). An analysis often yields many parameter value sets ("solutions") that fit the data of one experiment nearly equally well. In other words, the information content of the experiment is insufficient to resolve the parameters into a unique set of most probable values. A practical method to overcome this problem is simultaneous nonlinear regression (SNLR) (Kukkar et al., 2000) where the regression process is conducted for different experiments simultaneously and the modeling function shares parameters that are independent of the specific experimental condition. SNLR can be successful when complete data sets (with replicates) are obtained from experiments performed under different conditions as, for exam-
ple, in presence and absence of inhibitors of transport processes. Herein, SNLR was performed with average data values (with five hearts in each group: control, verapamil, and amiodarone) by using maximum likelihood estimation. Note that the model equations were identical up to factors $f_i$ characterizing selected transport parameters $P_i$ in the presence of Pgp inhibitors. The following information provided by ADAPT was used to evaluate the goodness of fit and the quality of parameter estimates: coefficients of variations of parameter estimates (CVs), parameter correlation matrix, sums of squares of residuals, visual examination of the distribution of residuals, and Akaike information criterion. As criteria for evaluating the numerical identifiability of estimates, we used CV < 0.5 and a correlation coefficient threshold of 0.9.

We started with SNLR of the idarubicin venous outflow concentration data obtained during a control perfusion and when Pgp antagonists were added to the perfusion, i.e., three sets of average data “control,” “verapamil,” and “amiodarone” were fitted simultaneously, whereby factors $f_i$ accounted for a potential change in parameters due to the presence of Pgp inhibitor. Then, the pharmacokinetic parameters were fixed in fitting the effect data to estimate $\tau_{\text{diff}}, E_{\text{kin}}, E_{\text{max}}$, and $N$ by using the same error model (eq. 8). If necessary, the model was modified and the process repeated until the model and the measured data were concordant in both cases.

**Statistics.** The outflow data are presented as average ± S.E. The significance of changes in the time course of outflow concentration in the presence of Pgp inhibitors was tested by repeated measures analysis of variance. $P$ values of less than 0.05 were considered statistically significant. Because in the present case global analysis (SNLR) can be performed only with pooled data, asymptotic standard errors of parameter estimates were obtained by nonlinear regression, which are generally over-optimistic. Thus, the likelihood ratio test (Huet et al., 1996) was used to determine the significance of parameter changes in the nested models due to the presence of verapamil or amiodarone.

**Results**

**Uptake Kinetics.** Figure 1A shows the average outflow concentration-time profiles obtained for the 10-min infusion of idarubicin in the absence and presence of the Pgp inhibitors verapamil or amiodarone ($n = 5$ independent experiments in each group). After reaching their peaks at the end of infusion, the curves decayed rapidly within 10 s, followed by a slow decline. Compared with control, the outflow concentration curve was shifted downward during the infusion pe-
period in the presence of verapamil (1 nM) or amiodarone (1 μM) (P < 0.05), indicating increased uptake of idarubicin. A series of different compartment models describing the myocardial kinetics of IDA was evaluated (e.g., possible alternative models with factors $f_{\text{Ver},12}$, etc., were tested). The best model selected according to the criteria described above is schematically shown in Fig. 2. In this model, idarubicin is transported from the extracellular space (compartment 1) to two intracellular compartments (2 and 3); the effect site does not influence mass balance in the system but $x_E(t)$ is delayed regarding $x_E(t)$ by a response time constant $\tau_{\text{eff}}$. Figure 2, B and C, show the resulting simultaneous fit of average idarubicin outflow concentration-time profiles for the three groups. The parameter estimates describing cardiac disposition of idarubicin in the absence and presence of Pgp inhibitors are listed in Table 1. The compartment model and the measured data are concordant. The apparent distribution volume of compartment 1 (14 ml) is larger than the distribution volume of an intravascular indicator, indicating rapid equilibration with a tissue region surrounding the vascular space. The saturable, Michaelis-Menten-type uptake process into the heart is characterized by the apparent maximal transport rate $V_{\text{max},12}$ and the apparent Michaelis constant $K_{m,12}$ ($x_1$ at which this uptake rate is at its half-maximal level) equal to 644 nmol/min and 42.8 nmol, respectively (alternatively, 46.0 μM/min and 3.06 μM in terms of concentration $C_1 = x_1/V_1$). Kinetic analysis of the data indicated the existence of a second carrier-mediated transport process that governs distribution from compartment 2 to compartment 3 ($V_{\text{max},23} = 347$ nmol/min, $K_{m,23} = 215$ nmol). The sequestration rate constant, $k_{\text{sec}}$, accounts for quasi-irreversible binding and metabolism of idarubicin. Pgp inhibition caused a nearly 2-fold increase in the maximal uptake rate ($P < 0.01$), i.e., an increase in $V_{\text{max},12}$ by factors $f_{\text{max},12,\text{vera}} = 1.76$ and $f_{\text{max},12,\text{amio}} = 1.80$ for verapamil and the effect site amount, respectively. These factors completely described the effect of verapamil and amiodarone on pharmacokinetics of idarubicin because all data were simultaneously fitted by a single set of parameter values (Table 1). The cardiac kinetics of idarubicin is characterized by high intracellular accumulation, with a predicted steady-state partition ratio for linear kinetics $k_{\text{out}}/k_{\text{in}} = (V_{\text{max},12}/K_{m,12})/k_{\text{eff}}$ of 5.6 (which would increase nearly 2-fold in the presence of Pgp inhibitors).

The estimated parameters of the variance model (eq. 8) show that the error has a standard deviation of about 10% of the measured concentration. Asymptotic coefficients of variation for the parameter estimates and approximate correlation coefficients were mostly less than 25% and 0.8, respectively. Only the parameters $V_1$ and $K_{m,23}$ are relatively "poorly determined" (Table 1).

**Negative Inotropic Effect.** Idarubicin (0.5 mg) decreased myocardial contractility (LVDP, $LVD_{\text{P/dt}_{\text{max}}}$), with maximum effects at the end of infusion. The LVDP and $LVD_{\text{P/dt}_{\text{max}}}$ (data not shown) were decreased to 48.7 and 51.2% of baseline level, respectively, and recovered within 30 min. Figure 3, A to C, display the time course of observed and model-predicted negative inotropic action, $E(t)$, of idarubicin. The effect as a function of drug amount at the effect site is depicted for the three experimental groups in Fig. 3D and the parameters are listed in Table 1. The response was described adequately by the $E_{\text{max}}$ model with higher sigmoidicity ($N \approx 2$) in the presence of verapamil and amiodarone (a Hill coefficient did not improve the fit in the control group). Both Pgp inhibitors attenuated the negative inotropic effect of idarubicin, shifting the amount (or concentration)-effect relationship downwards (the ratio $E_{\text{max}}/E_{\text{50}}$ decreased from 2.8 to 1.6%/μg). The equilibration time $\tau_{\text{eff}}$ of 0.52 min between cytosolic idarubicin concentration and effect increased more than 3-fold in the presence of Pgp inhibitors. Alternative models assuming either a time-dependent signal transduction (Mager and Jusko, 2001) or a reduced $E_{\text{max}}$ model, $E \sim C^n$ (Paschoa et al., 1998), failed to fit the data.

**Discussion**

This study provides evidence that 1) uptake of the lipophilic anthracycline idarubicin in rat heart is through a saturable transport process, 2) verapamil and amiodarone increase the maximal uptake rate, and 3) intracellular kinetics of idarubicin is closely related to its negative inotropic action whereby both Pgp inhibitors attenuate the cellular concentration-response relationship.

Uptake kinetics of anthracyclines, including idarubicin, has previously been studied in various cell lines; however, the transport mechanism is not completely clear. In the present study, we investigate for the first time the uptake kinetics of a Pgp substrate into the intact heart. The observed nearly 2-fold increase in $V_{\text{max},12}$ by Pgp antagonists suggests that under control conditions Pgp pumps idarubicin out of the membrane, thus limiting its uptake (Fig. 2). The observed (net) rate of myocardial idarubicin uptake represents a balance between two opposing processes: active pumping by Pgp back to the extracellular space and saturable transport of drug across the membrane. Our finding is consistent with the "hydrophobic vacuum cleaner" model (Gottesman and Pastan, 1993), where Pgp interacts directly with substrates in the plasma membrane accounting for decreased drug influx rates (Pgp removes drugs directly from the membrane), i.e., Pgp substrates probably gain access to...
the protein after partitioning into the membrane rather than from the aqueous phase. The fact that only $V_{\text{m,12}}$ but not $K_{\text{m,12}}$ is affected suggests a noncompetitive interaction in accordance with the interaction between anthracyclines and verapamil in MDR cells (Nielsen et al., 1995; Stein, 1997). It has been shown that Pgp is expressed in heart tissue (van der Valk et al., 1990; Cayre et al., 1996; Beaulieu et al., 1997; Estevez et al., 2000), and that Pgp has a role in the cardiac uptake of anthracyclines was recently suggested by pharmacokinetic studies in mice lacking mdr1a Pgp (mdr1a (−/−) mice) (van Asperen et al., 1999b). The quantitative analysis of uptake kinetics after a 10-min infusion of idarubicin described herein extends considerably our previous observation that verapamil decreased the recovery of idarubicin in a washout experiment after 1-min infusion (Kang and Weiss, 2001). That Pgp inhibition primarily increased cardiac uptake but not efflux from the heart ("influx hindrance") is in accordance with findings by Decleves et al. (1998) in leukemia HL-60 cells, who suggested that intracellular trapping of molecules could limit their availability for Pgp-mediated efflux. In contrast to the brain, where the Pgp pump is located on the luminal side of the capillary endothelial cell (Beaulieu et al., 1997), its location is less clear in the heart. Although Pgp is present in the plasma membrane of cardiomyocytes (Cayre et al., 1996; Estevez et al., 2000) and our kinetic-dynamic model indicates that compartment 2 represents the cytosol (see below), we cannot differentiate between capillary wall and sarcolemma as permeability barriers.

The observation of Michaelis-Menten kinetics for uptake of the hydrophobic compound idarubicin in the intact heart is an interesting, novel result because the underlying mechanism is still poorly understood. There seems to be contradicting and confusing evidence in the literature with regard to anthracycline transport in cancer cell lines. On the one hand, it is suggested that passive membrane permeation plays a substantial role in controlling cellular accumulation together with Pgp-mediated efflux (for review, see Stein, 1997; Wielinga et al., 2000). On the other hand, saturable uptake transport of anthracyclines into normal and MDR cells has been reported by several groups (Skovsgaard, 1978; Kerr et al., 1986; Usansky et al., 1991; Nielsen et al., 1995; Nagasawa et al., 1996, 1997; Decorti et al., 1998; Sasaya et al., 1998). Thus, in human mononuclear cells and HL-60 cells, uptake of idarubicin was primarily carrier-mediated with a contribution of passive diffusion of about 10% (Nagasawa et al., 1997). Similar to our results, verapamil in MDR cells affected preferentially influx of daunorubicin increasing $V_{\text{m,12}}$ by about 50% (Nielsen et al., 1995). However, the discussion of the underlying mechanisms is controversial (Kerr et al., 1986; Nielsen et al., 1995; Decorti et al. (1998) and Sasaya et al. (1998) suggested that saturation of nonspecific binding sites on kidney epithelial cells could mimic carrier-mediated transport. The role of the intracellular transporter with lower capacity ($V_{\text{m,23}} \approx 0.5 \ V_{\text{m,12}}$) and affinity ($K_{\text{m,23}} \approx 5$ $K_{\text{m,12}}$) is less clear. One may speculate that compartment 3 represents a subcellular pool, e.g., cytoplasmic organelles (Larsen et al., 2000), but in contrast to the uptake transporter, the estimation of the parameters $V_{\text{m,23}}$ and $K_{\text{m,23}}$ was less reliable (see below). Figure 1D shows that more than 90% of the idarubicin amount that was taken up remains in the heart (or is metabolized) after the 80-min washout period, demonstrating the strong binding to intracellular constituents (DNA) and trapping in acidic vesicles.

The maximum decrease in LVDP was comparable to that observed for the 1-min infusion of 0.5 mg of idarubicin (Kang and Weiss, 2001). Although it has been suggested that the acute cardiac effects of anthracyclines are due to impaired myocardial Ca$^{2+}$ handling, the mechanism appears still unclear (Matsushita et al., 2000). Several studies indicated that anthracyclines activate the cardiac sarcolemmal reticulum Ca$^{2+}$ release channel (ryanodine receptor), leading to an intracellular Ca$^{2+}$ overload associated with an impairment of Ca$^{2+}$ transients and a decrease in myocardial contractility (Holmberg and Williams, 1990; Temma et al., 1994, 1996; Maeda et al., 1999). Recently, it has been suggested that this negative inotropic action could also result from an inhibition of sarcolemmal reticulum Ca$^{2+}$ release (Olson et al., 2000). A dose-dependent decrease in the amplitude of cytosolic Ca$^{2+}$

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**TABLE 1**

Model parameter estimates for the pharmacokinetics and pharmacodynamics of IDA in hearts from control, verapamil- (1 nM), and amiodarone- (1 μM) treated groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV%</th>
<th>Parameter</th>
<th>Estimate</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{m,12}}$ (nmol/min)</td>
<td>644</td>
<td>18</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f_{\text{max,12,vera}}$</td>
<td>1.76</td>
<td>3</td>
<td>$\tau_{\text{off}}$ (min)</td>
<td>0.52</td>
<td>18</td>
</tr>
<tr>
<td>$f_{\text{max,12,min}}$</td>
<td>1.80</td>
<td>3</td>
<td>$E_{\text{m,12}}$ (%)</td>
<td>54.1</td>
<td>4</td>
</tr>
<tr>
<td>$K_{\text{m,12}}$ (nmol)</td>
<td>42.8</td>
<td>17</td>
<td>$E_{\text{m,12}}$ (µg)</td>
<td>19.2</td>
<td>7</td>
</tr>
<tr>
<td>$K_{\text{m,23}}$ (min$^{-1}$)</td>
<td>2.6</td>
<td>26</td>
<td>Verapamil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$k_{\text{m,12}}$ (min$^{-1}$)</td>
<td>0.55</td>
<td>17</td>
<td>$\tau_{\text{off}}$ (min)</td>
<td>1.67</td>
<td>4</td>
</tr>
<tr>
<td>$V_{\text{m,23}}$ (nmol/min)</td>
<td>347</td>
<td>23</td>
<td>$E_{\text{m,23}}$ (%)</td>
<td>65.4</td>
<td>12</td>
</tr>
<tr>
<td>$K_{\text{m,23}}$ (nmol)</td>
<td>215</td>
<td>44</td>
<td>$E_{\text{m,23}}$ (µg)</td>
<td>40.8</td>
<td>11</td>
</tr>
<tr>
<td>$k_{\text{m,23}}$ (min$^{-1}$)</td>
<td>0.22</td>
<td>8</td>
<td>$N_{\text{m,23}}$ (%)</td>
<td>2.3</td>
<td>9</td>
</tr>
</tbody>
</table>

Amiodarone | | |
| $\sigma_{\text{m,12}}$ (µg/ml) | <10$^{-7}$ | | $\tau_{\text{off}}$ (min) | 2.36 | 6 |
| $\sigma_{\text{cont}}$ | 0.14 | | $E_{\text{m,12}}$ (%) | 45.9 | 9 |
| $\sigma_{\text{vera}}$ | 0.12 | | $E_{\text{m,23}}$ (%) | 28.6 | 10 |
| $\sigma_{\text{amio}}$ | 0.09 | | $N_{\text{m,23}}$ (%) | 2.4 | 11 |

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$^a$ Simultaneous nonlinear regression of three IDA outflow concentration-time profiles (mean curves of control, verapamil, and amiodarone group, see Fig. 1, B and C), where one additional parameter in the model equations accounted for the effect of verapamil ($f_{\text{max,12,vera}}$) or amiodarone ($f_{\text{max,12,min}}$), respectively (e.g., $V_{\text{m,12,vera}} = f_{\text{max,12,vera}} V_{\text{m,12}}$).

$^b$ Final estimates (fit of LVDP data) with fixed pharmacokinetic parameters.

$^c$ Asymptotic coefficient of variation.
transients has been observed for idarubicin in isolated rat myocytes (P. Wolna and M. Weiss, unpublished data). The present finding that the effect \( E(t) \), i.e., the decrease in contractility, is linked to the time course of idarubicin in compartment 2, \( x_2(t) \), with a relatively short equilibration time constant of 0.52 min appears consistent with this concept (Fig. 3A). Suggesting that \( x_2(t) \) is the amount in the pharmacologically active pool, the kinetic-dynamic modeling sheds light on the possible anatomical/physiological role of the compartments, where compartment 2 may represent the cytosol with trans-sarcolemmal idarubicin influx from compartment 1. The model analysis allows, for the first time, a separation of the kinetic and dynamic effects determining the verapamil/amiodarone-idarubicin interaction. Theoretically, both cardioactive drugs interfere with stimulus-contraction coupling. The attenuation of the idarubicin-induced negative inotropic effect (Fig. 3D) is in accordance with the protective effects of a calcium antagonist on doxorubicin-induced impairment of \( \text{Ca}^{2+} \) transients in rat cardiac myocytes (Maeda et al., 1999) and the inhibitory effect of amiodarone on the \( \text{Na}^+ / \text{Ca}^{2+} \) exchanger, which has been suggested to prevent \( \text{Ca}^{2+} \) overload under pathological conditions (Watanabe and Kimura, 2000). However, because the complex mechanism of the negative inotropic action of idarubicin is not well understood and the available data are very limited, our empirical model is necessarily an oversimplification, which, for example, does not explain the physiological role of \( \tau_{\text{eff}} \). Because only 2% of the idarubicin dose were metabolized in the heart (up to 80 min after 1-min infusion) to the active metabolite idarubicinol (Kang and Weiss, 2001), metabolite effects were neglected in the analysis.

With regard to model identifiability and the precision of parameter estimates, it is encouraging that the approximate coefficients of variation and correlation coefficients between parameters were <50% and <0.9, respectively. Furthermore, a kinetic model will gain credibility if it can describe experimental data under different conditions (absence and presence of Pgp inhibitors) by adjustment of only one parameter (\( V_{\text{max},12} \) for uptake transport). Because to our knowledge this is the first report suggesting saturable uptake of a hydrophobic compound at the organ level, it is important to note that all tested alternative models based on passive influx completely failed to fit the data. The information obtained from idarubicin pharmacodynamics was necessary to identify the intracellular distribution kinetics, i.e., to obtain initial estimates of \( V_{\text{max},23} \) and \( K_{\text{m},23} \). As also was obvious from the
higher approximate coefficient of variation of $K_{\alpha,23}$. We obtained less experimental evidence in support of this intracellular uptake transporter. In view of the relatively slow drug input rate used in this experiment, it is not surprising that the initial distribution volume $V_i$ was not uniquely identifiable. The value of 14.0 ml represents the optimal estimate, which was then fixed to obtain the approximate CVs for the other parameters (Table 1). (Note that the apparent extracellular distribution volume $V_e$ also accounts for rapid binding processes and has no direct anatomical meaning.) The necessarily simplified approach taken herein represents a useful “minimal” heart model (Weiss, 1998); however, as any model, it does not provide a unique picture and must evolve with newly acquired data and knowledge.

It is concluded that the cardiac uptake of idarubicin is saturable and that the uptake rate is increased by verapamil and amiodarone, probably because of impairment of Pgp-mediated influx hindrance. Although we have used idarubicin as a hydrophobic model compound, these findings should be relevant to other Pgp substrates. In addition, the combined kinetic-dynamic model provided further insight into the mechanism underlying the time course of the acute negative inotropic effect of anthracyclines.

References


References


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