Pharmaceutical Research, Vol. 24, No. 1, January 2007 (© 2006)
DOI: 10.1007/s11095-006-9117-z

Research Paper

Modeling Cardiac Uptake and Negative Inotropic Response of Verapamil in Rat Heart: Effect of Amiodarone

Pakawadee Sermsappasuk,[1] Osama Abdelrahman,[1] and Michael Weiss[1,2]

Received May 10, 2006; accepted July 10, 2006; published online September 13, 2006

Purpose. To determine the effect of the P-glycoprotein (Pgp) modulator amiodarone on the pharmacokinetics and pharmacodynamics (PK/PD) of Pgp substrate verapamil in the perfused rat heart.
Methods. In Langendorff-perfused rat hearts, the outflow concentration–time curve and inotropic response data were measured after a 1.5 nmol dose of [^{3}H]-verapamil (infused within 1 min) in the absence and presence of the amiodarone (1 μM) in perfusate, as well as using a double dosing regimen (0.75 nmol in a 10 min interval). These data were analyzed by a PK/PD model.
Results. Amiodarone failed to influence the rapid uptake and equilibrium partitioning of verapamil into the heart. The time course of the negative inotropic effect of verapamil, including the 'rebound' above the original baseline after the infusion of verapamil was stopped, could be described by a PK/PD tolerance model. Tolerance development (mean delay time, 12 min) led to a reduction in predicted steady-state effect (16%). The EC_{50} and E_{max} values as estimated in single dose experiments were 16.4 ± 4.1 nM and 50.5 ± 18.9 mmHg, respectively.
Conclusions. The result does not support the hypothesis that Pgp inhibition by amiodarone increases cardiac uptake of the Pgp substrate verapamil.

KEY WORDS: heart; p-glycoprotein; pharmacokinetic/pharmacodynamic model; tolerance; verapamil.

INTRODUCTION

Verapamil causes vasodilation and depresses myocardial contractility and electrical activity in the atrioventricular and sinoatrial nodes through inhibition of the L-type calcium channels (1,2). Calcium antagonists are used in the treatment of stroke, hypertension, cardiac arrhythmias and angina pectoris (3). Although the use of verapamil may be limited by its direct negative inotropic effect, less is known on its cardiac uptake kinetics in relation to the time course of the negative inotropic action and factors which affect these processes (4,5).

Verapamil is also a standard substrate of P-glycoprotein (Pgp); however in contrast to the brain [e.g., (6)], the functional role of this transporter for drug uptake into the myocardium is not well established [for review see Ref. (7)]. In the heart, Pgp is expressed in endothelial cells of capillaries (8) but not in cardiomyocytes (9). However, the level is far below those detected in the liver, kidneys, and brain (10). An increased cardiac accumulation of doxorubicin has been reported in mdr1a Pgp deficient mice (11). We have previously interpreted the substantial increase in myocardial uptake of the Pgp substrate idarubicin in the presence of verapamil or amiodarone in terms of an impairment of Pgp-mediated influx hindrance (12); however, this hypothesis remains open to debate in view of alternative explanations in terms of physico-chemical properties of these drugs (13), including the membrane transport of idarubicin by a flip-flop mechanism (14). Since cardiac Pgp pumps may alter intracardiac concentrations and hence the efficacy and toxicity of cardioactive drugs, an understanding of their role in myocardial drug uptake merits further investigations (7).

Therefore, the objectives of the present investigation were: 1) to analyze uptake and inotropic response of verapamil in the perfused rat heart using a pharmacokinetic–pharmacodynamic (PK/PD) modeling approach that accounts acute tolerance to the negative inotropic verapamil effect, and 2) to evaluate the effect of the Pgp inhibitor amiodarone (15) on the cardiac uptake kinetics and negative inotropic response of verapamil.

MATERIALS AND METHODS

Drugs

Verapamil hydrochloride, [*N*-methyl-^{3}H]verapamil hydrochloride (80 Ci/mmol), amiodarone hydrochloride and dimethyl sulfoxide were purchased from MP Biomedicals

[1] Section of Pharmacokinetics, Department of Pharmacology, Martin Luther University Halle-Wittenberg, D-06097, Halle, Germany.
[2] To whom correspondence should be addressed. (e-mail: michael.weiss@medizin.uni-halle.de)

ABBREVIATIONS: CVR, coronary vascular resistance; LVDP, left ventricular developed pressure; Pgp, P-glycoprotein; PK/PD, pharmacokinetic/pharmacodynamic.

(Eschwege, Germany), American radiolabeled chemicals (St. Louis, USA), Sigma–Aldrich chemie (Steinheim, Germany) and Carl Roth (Karlsruhe, Germany), respectively. All other chemicals and solvents were of highest grade available.

Perfused Rat Heart

Male Wistar rats weighing 280 to 320 g were heparinized and anesthetized with pentobarbital. Once the rat was anesthetized, the heart was rapidly removed into oxygenated ice-cold modified Krebs–Henseleit buffer. Hearts were transferred to the Langendorff perfusion apparatus where they were perfused retrogradely with a Krebs–Henseleit buffer containing NaCl (118 mM), KCl (4.7 mM), $CaCl_2$ (1.5 mM), $MgSO_4$ (1.66 mM), $NaHCO_3$ (24.88 mM), KH_2PO_4 (1.18 mM), glucose (5.55 mM), Na-pyruvate (2 mM), and bovine albumin (0.1%w/v) and equilibrated with 95% CO_2 and 5% O_2 at 37°C. After stabilization, the system was changed to constant flow condition, maintaining a coronary flow of 9.7 ± 0.5 ml/min. A latex balloon tied to the end of a polyethylene tube was passed into the left ventricle through the mitral valve, and it was connected to a pressure transducer. The balloon was inflated with 50% methanol to create a diastolic pressure of 5 to 6 mmHg. The hearts were beating spontaneously at an average rate of 252 ± 17 beats/min. Coronary perfusion pressure, the left ventricular pressure, and heart rate (HR) were measured continuously and a physiological recording system (Hugo Sachs Elektronik, March–Hugstetten, Germany) was used to monitor left ventricular systolic pressure (LVSP) and left ventricular end diastolic pressure (LVEDP). Left ventricular developed pressure (LVDP) is defined as LVDP = LVSP − LVEDP. Coronary vascular resistance (CVR) is calculated from perfusion pressure divided by coronary flow. This investigation conformed to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85–23, Revised 1996). Prior approval was obtained by the Animal Protection Body of the State of Sachsen-Anhalt, Germany.

Experimental Protocol

The verapamil solution infused in single dose (1.5 nmol), and double dosing experiments (0.75 nmol) was firstly prepared by diluting from a concentrated stock solution in water (2.5 mM) into the perfusate buffer. Then, the 3 ml of the diluted solution were mixed with 2 µl of a labeled verapamil (1 mCi/ml). The final labeled solution was, finally, infused into the aortic cannula directly above the heart.

In single dose control experiments (eight hearts), after a 20-min equilibration period, the labeled 1.5 nmol verapamil was infused for 1 min and outflow samples were collected every 5 s for 3 min, every 10 s for next 7 min, every 30 s for next 10 min, and every 1 min for the next 5 min. The double dosing experiment (eight hearts) was conducted by infusing the first dose of labeled 0.75 µM verapamil for 1 min at time 0, defined as 20 min after the start of experiment, and the second dose 10 min later. The samples were frozen at −20°C until analysis. The sample analysis was conducted within 3 days after collecting samples using a liquid scintillation counter (Perkin Elmer Instruments, Shelton, CT). In another series of single dose experiments (four hearts), the same procedure was conducted in presence of amiodarone (1 µM) in perfusate. The perfusate containing 1 µM amiodarone was prepared by diluting a concentrated 2 mM amiodarone in DMSO solution. The final concentration of DMSO was ≤0.05%. This DMSO concentration had no significant inotropic effect.

Initiated by the observation of an unexpected rebound phenomenon, a cumulative infusion of verapamil with input concentrations of 7.4, 14.9, 22.3, 29.7, 49.6, 74.3, 99.0, and 247.9 nM (each infusion for 15 min) was performed in four hearts.

PK/PD Model and Data Analysis

The model of cardiac PK of verapamil (Fig. 1) describes drug uptake from the vascular space (V_{vas}) into the tissue compartment with an uptake rate k_{in}, where $k_{in} = CL_p/V_{vas}$ and CL_p is the permeation clearance or permeability–surface product. In fitting the outflow data, this parameter was fixed to the value $V_{vas} = 0.06$ ml/g, taken anatomic from data (16). Note that perfusate flow (flow rate Q) and drug input (rate QC_{in}) as well as drug outflow (rate constant Q/V_{vas}) occur in this vascular compartment. An additional compartment with volume V_0 (in series with the vascular compartment) was introduced (not shown in Fig. 1) to account for the mixing in nonexchanging elements of the system. Tissue distribution is

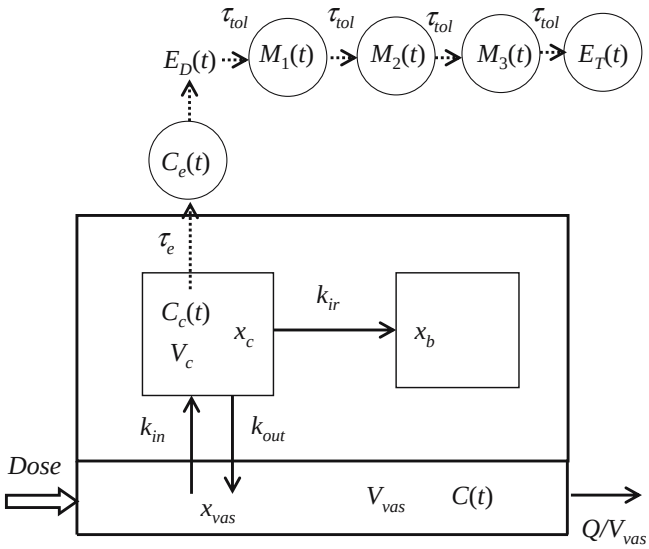

Fig. 1. Model of verapamil pharmacokinetics and pharmacodynamics in the perfused rat heart. The rate constants, k_{in} and k_{out}, describe uptake from the vascular space (V_{vas}) into the heart (permeation clearance, $CL_p = k_{in}V_{vas}$) where tissue concentration $C_c(t)$ is governed by the distribution volume V_c. The direct negative inotropic effect $E_D(t)$ (decrease in LVDP) is related to the delayed (time constant τ_e) effect site concentration $C_e(t)$ by a sigmoidal E_{max} model, and causes a compensatory positive inotropic tolerance effect $gE_T(t)$ $(0 < g < 1)$ that is delayed with respect to $E_D(t)$ by a mean delay time of $4\tau_m$ Eq. (10). The net effect is given by $E(t) = E_0 - E_D(t) + gE_T(t)$ Eq. (6).

characterized by the distribution volume accounting for reversible quasi-instantaneous myocardial binding $V_c = (k_{in}/k_{out})V_{vas}$. The rate constants k_{ir} describes irreversible tissue binding (during the time course of experiment) and/or metabolism of verapamil. Neglecting this effect of quasi-irreversible binding, the total (or steady-state) distribution volume of the heart is then given by

$$V_{ss} = V_{vas}(1 + k_{in}/k_{out}) \quad (1)$$

and the equilibrium cardiac tissue/perfusate partition coefficient of verapamil is obtained as $K_{pu} = V_{ss,\,vera}/V_{ss,\,water}$, where $V_{ss,\,water}$ denotes the water distribution volume of the rat heart.

The changes in drug amounts (x_i) in compartments i after input (dosing) rate R are described by the following differential equations

$$\frac{dx_0(t)}{dt} = -\frac{Q}{V_0}x_0(t) + R \quad (2)$$

$$\frac{dx_{vas}(t)}{dt} = \frac{Q}{V_0}x_0(t) + k_{out}x_c(t) - k_{in}x_{vas}(t) - \frac{Q}{V_{vas}}x_{vas}(t) \quad (3)$$

$$\frac{dx_c(t)}{dt} = k_{in}x_{vas}(t) - k_{out}x_c(t) - k_{ir}x_c(t) \quad (4)$$

$$\frac{dx_b(t)}{dt} = k_{ir}x_c(t) \quad (5)$$

Note that the measured outflow concentration $C_{out}(t) = x_{vas}(t)/V_{vas}$ is the concentration in the vascular compartment.

Since the inotropic effect rebounded to levels above the original baseline after the verapamil infusion was stopped (Fig. 2B), the cardiac PK/PD model previously used for amiodarone (17) was extended to account for the tolerance development. Analogous to the tolerance model by Mandema and Wada (18), the observed effect was regarded as the sum of the baseline effect E_0 (average pre-dose value), the 'direct' negative inotropic effect $E_D(t)$, and a compensatory positive inotropic effect $E_T(t)$ (Fig. 1)

$$E(t) = E_0 - E_D(t) + gE_T(t) \quad (6)$$

where the constant g determines the extent of tolerance development. Like in the normal link model without tolerance, $E_D(t)$ is related to the effect site (myocardial) concentration $C_c(t)$ via a sigmoid E_{max} model (Hill equation)

$$E_D(t) = \frac{E_{max}C_e^N(t)}{EC_{50}^N + C_e^N(t)} \quad (7)$$

where EC_{50} is the effect concentration that corresponds to 50% of the maximum effect (E_{max}) and N is the Hill coefficient that determines the sigmoidicity of the curve. The effect site concentration $C_e(t)$ in Eq. (7) is delayed (time constant τ_e) relative to the myocardial concentration $C_c(t)$

$$\frac{dC_e(t)}{dt} = \frac{1}{\tau_e}[C_c(t) - C_e(t)] \quad (8)$$

We assumed that the negative inotropic effect of verapamil $E_D(t)$ triggers a counter-regulation, i.e., the delayed positive inotropic response $E_T(t)$. This delay time of the tolerance effect $E_T(t)$ with respect to $E_D(t)$ was not simply due to a first-order process as in previously used tolerance models (18), but could be well approximated by a gamma distribution with n = 4, i.e., a series of transit compartments (19)

$$\frac{dM_i(t)}{dt} = \frac{1}{\tau_m}[M_{i-1}(t) - M_i(t)] \qquad \text{for } i{=}1\ldots4 \quad (9)$$

where $M_0(t) = E_0 - E_D(t)$ and $M_4(t) = E_T(t)$. Note that the mean delay time of the tolerance effect MTT_{tol} (i.e., the mean transit time of the gamma distribution) is given by

$$MTT_{tol} = n\tau_m = 4\tau_m \quad (10)$$

and $1/MTT_{tol}$ characterizes the rate of tolerance development. At steady-state, we obtain from Eqs. (6) and (9)

$$\Delta E_{ss}/E_{ss,\,non-tolerance} = g \quad (11)$$

Thus, tolerance development attenuates the negative inotropic effect by the fraction g and it becomes clear that $0 < g < 1$. As a measure of inotropic response $E(t)$, we used the time course of left ventricular developed pressure LVDP(t), i.e., E_0 is identical to the baseline (predrug) contractility $LVDP_0$.

Equations (2) to (9) were solved numerically and fitted to the data using ADAPT II Version 4 (20). First the outflow concentration data were fitted to estimate the PK parameters. These parameter values were then held fixed in fitting Eqs. (6), (7) and (9) to the $E(t)$ data. Using maximum likelihood estimation, we assumed that the measurement error has a standard deviation which is a linear function of the measured quantity. The 'goodness of fit' was judged by visual examination of the distribution of residuals and Akaike information criterion (AIC value); additionally, the R^2 value of the fits was reported. The reliability of parameter estimation was assessed by the asymptotic coefficients of variation (CV) of individual parameter estimates.

The cumulative infusion data relating inflow concentration of verapamil to inotropic effect measured after 15 min were directly fitted by Eq. (7) to estimate the PD parameters.

The recovery of verapamil was calculated from the inflow and outflow concentration *versus* time data using a numerical integration method as the amount recovered at the end of experiment (t_{last} = 25 min):

$$\text{Recovery} = \frac{\int_0^{25} C_{out}(t)dt}{Dose} \quad (12)$$

The results are expressed as mean ± SD of the parameters estimated in the control single-dose (n = 8), control double-dose (n = 8) and amiodarone (n = 4) groups, respectively. Kruskal–Wallis ANOVA on ranks and Dunn all-pairwise comparison test were used to evaluate differences between group means. A p–value of less than 0.05 was considered statistically significant.

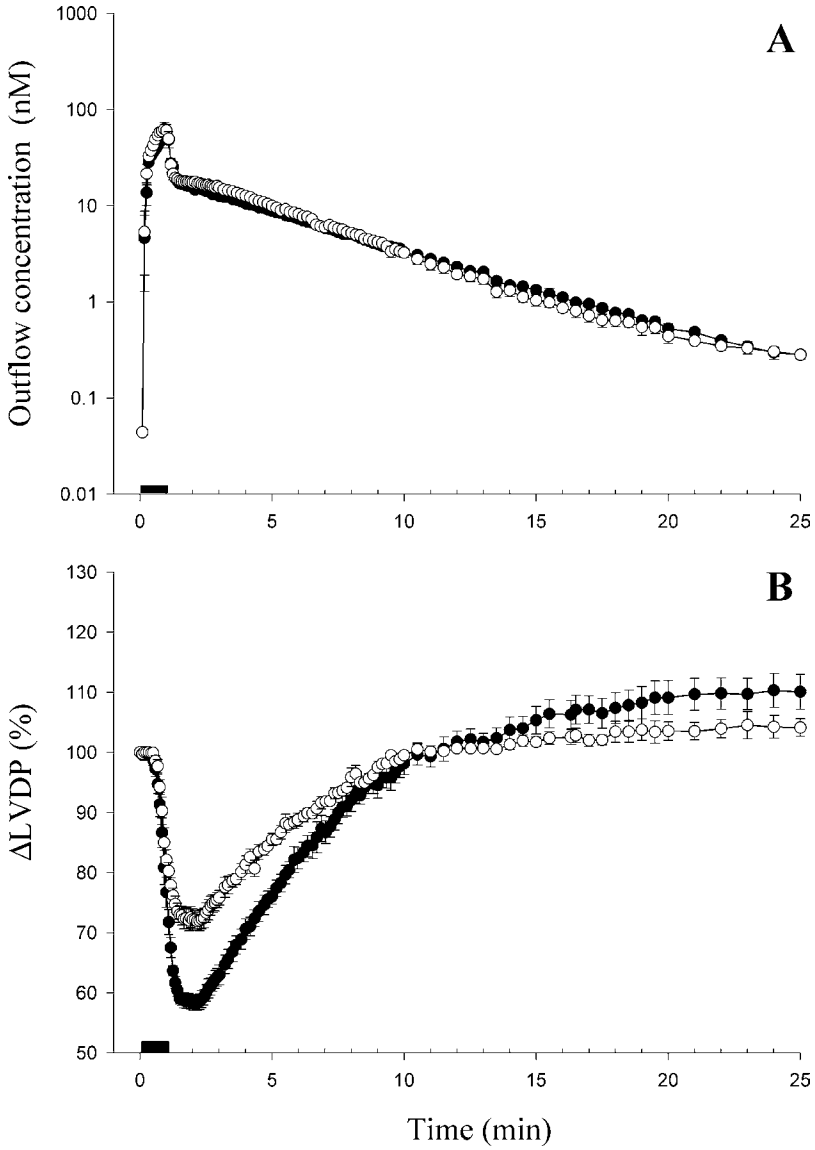

Fig. 2. (A) Outflow concentration–time profiles and (B) negative inotropic effect of verapamil (−LVDP in the percentage of baseline) in rat hearts for a 1-min infusion 1.5 nmol of verapamil, measured in control experiments (filled circle) and in the presence of 1 μM amiodarone in perfusate (open circle) (mean ± SEM). Error bars that fall within the symbols are not shown.

RESULTS

Figure 2A shows the mean outflow concentration-time profiles for the 1-min infusion of 1.5 nmol verapamil in absence (control) and presence of amiodarone (1 μM) in the perfusate. The recovery of verapamil in the perfusate (up to 25 min) for control and amiodarone group amounted to 89.2 ± 8.3% and 94.1 ± 12.2%, respectively. There were no statistical significant differences between both groups. The corresponding time course of negative inotropic effect is presented in Fig. 2B. Compared with the respective baseline values, the maximum negative inotropic effect of verapamil was 42.6 ± 3.1% and 28.1 ± 3.0% for control and amiodarone group, respectively ($p < 0.001$). There was a tendency towards a baseline reduction in the presence of amiodarone. Compared to the baseline before amiodarone infusion, inotropy was reduced by 14.3 ± 5.3%. The maximum effect was achieved at ~2 min after infusion in both groups, and the negative inotropic effect recovered to baseline after ~10 min. This was followed by a positive inotropic rebound effect in the control group that was nearly abolished in the amiodarone group. The coronary vascular resistance (CVR) of 4.67 ± 0.83 mmHg min/ml under control conditions was reduced by 42% to 2.73 ± 0.22 75 mmHg min/ml in the presence of amiodarone in perfusate ($p < 0.001$). Verapamil infusion induced a coronary vasodilation with a 17.9 ± 6.1% decrease in CVR ($p < 0.01$). Under vasodilation with amiodarone, only a 4.3 ± 1.6% further decrease in CVR was observed ($p < 0.01$).

The fit of the PK/PD model to outflow concentration and inotropic response data of verapamil after a single dose are exemplified in Fig. 3. Note that 'representative fits' means

that an experiment (heart) with an AIC value which was closest to group median value was selected.

The model well described the biphasic inotropic response: the negative inotropic effect followed by a rebound increase in contractility. The mean R^2, a measure of goodness of fit of the model, were 0.953 and 0.991 for PK and PD data, respectively. A comparable good fit and similar parameter estimates were obtained from the data of the double dose experiment where 10 min after the 1-min infusion of 0.75 nmol verapamil a second dose was given (Fig. 4). Also the single dose response in the presence of amiodarone in perfusate was well described by the PK/PD tolerance model (Fig. 5). The averaged model parameters and estimation errors (as coefficients of variation obtained in individual fits) are listed in Table I. Most parameters were estimated with sufficient precision, i.e., with relatively low asymptotic coefficients of variation. Exceptions are the parameters of the tolerance model (τ_m and g) in single dose experiments.

The uptake or permeation clearance of verapamil (CL_p = 38.1 ± 11.0 ml/min) was higher than perfusate flow (9.7 ml/min) indicating flow-limited uptake. The steady-state distribution volume of 51.2 ± 11.6 l corresponds to an equilibrium tissue/perfusate partition coefficient K_{pu} of 64.0 ± 14.5.

The 'direct' negative inotropic action of verapamil (−ΔLVDP) following a single dose was characterized by EC_{50} = 16.4 ± 4.1 nM, E_{max} = 50.5 ± 18.9 mmHg and a delay relative to the free myocardial concentration $C_c(t)$ of τ_e = 0.34 ± 0.15 min. Extent and delay of tolerance development were described by parameters g = 0.27 and MTT_{tol} = 25 min. These parameters were estimated with higher precision in double dose experiments (g = 0.16 and MTT_{tol} = 12 min). Although according to average effect–time curves (Fig. 2B) the rebound positive inotropic response appears to be nearly abolished in the presence of amiodarone, the tolerance model provided a better fit than normal PK/PD link model [g = 0 in Eq. (6)]. There was a tendency toward a reduced tolerance development (decrease in g and increase in

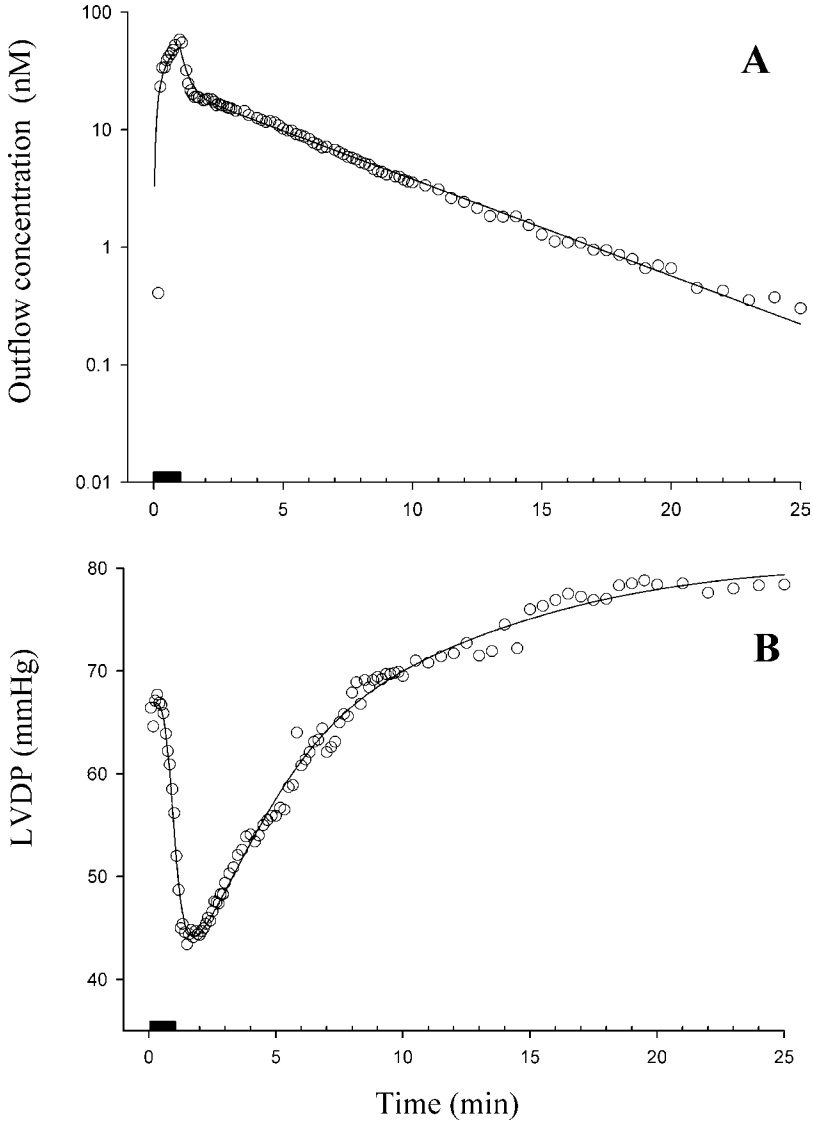

Fig. 3. Representative fits of the PK/PD tolerance model to experimental data obtained in one heart for a 1-min infusion 1.5 nmol of verapamil: (A) outflow concentration and (B) negative inotropic effect.

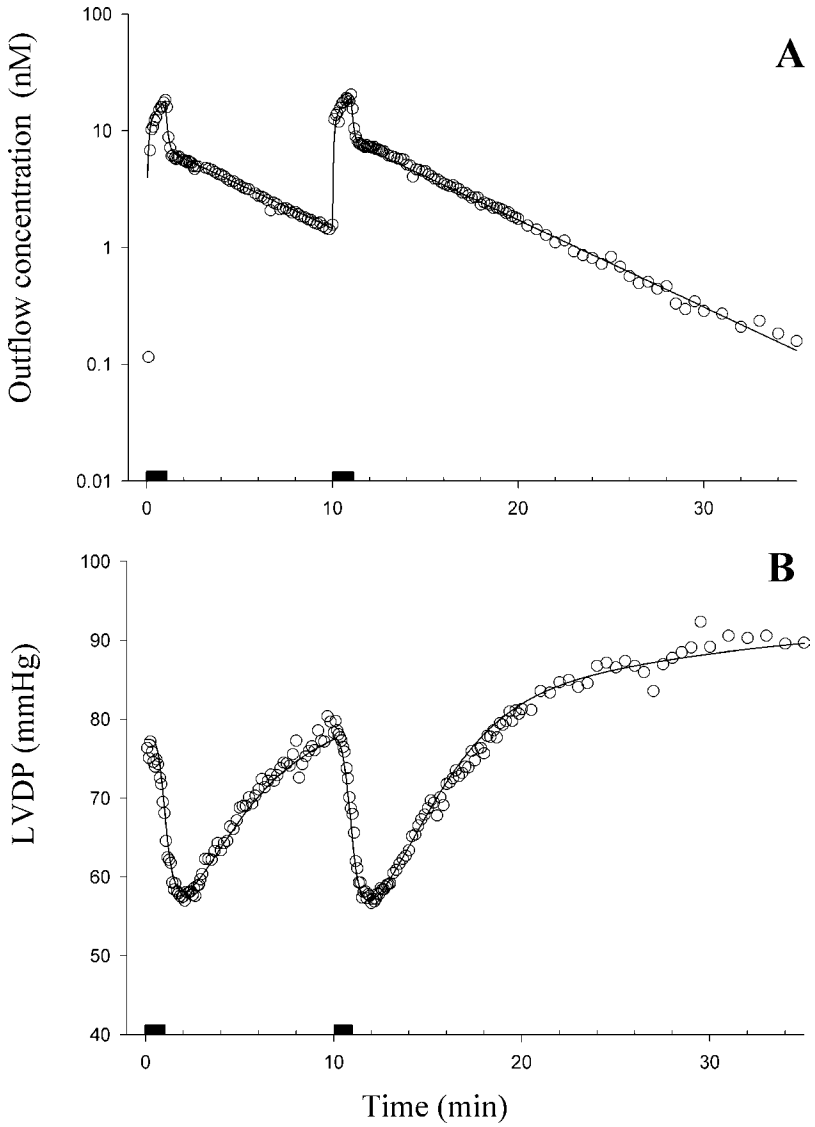

Fig. 4. (A) Representative fits of outflow concentration and (B) negative inotropic effect after two 0.75 nmol doses of verapamil in one heart.

MTT_{tol}) as well as a reduction in E_0 and E_{max} but the differences between groups were not significant.

The parameter estimates obtained by fitting Eq. (7) to the negative inotropic response following stepwise verapamil infusion were EC_{50} = 31.6 ± 14.2 nM, E_{max} = 73.2 ± 11.2% reduction, and N = 1.2 ± 0.5. The asymptotic coefficients of variation (CV) of individual parameter estimates were less than 13%.

DISCUSSION

Although verapamil, as most drugs, exerts its effects not within the plasma compartment but at receptors in target tissues, this distribution process to the site of action has not got much attention in the past. Here we analyzed the cardiac PK of verapamil and its relationship to negative inotropic response in perfused rat hearts under control conditions and in the presence of amiodarone. The latter was used in a concentration that significantly increased the cardiac uptake of idarubicin (12).

Pharmacokinetics

The lipophilic drug verapamil distributed rapidly into the rat heart with a permeation clearance CL_p (effective permeability-surface product) that is ~5-fold higher than that of digoxin or the hydrophilic solute sucrose (21). Note however that under *in vivo* conditions the degree of plasma protein binding has to be taken into account. Plasma protein binding of 90% (22) reduces CL_p to 10% of the estimated 38 ml/min. The equilibrium tissue/perfusate partition coefficient K_{pu} of 64 is in good agreement with K_p values of 5.1 (5) and 6.2 (23) measured in dogs for a free fraction in plasma f_u = 0.1 (since $K_p = f_u K_{pu}$). In order to elucidate the underlying cardiac binding sites, it appears especially interesting that K_{pu} values of 94.8 and 66.2 were theoretically predicted for rat heart using a mechanistic equation that takes partitioning into

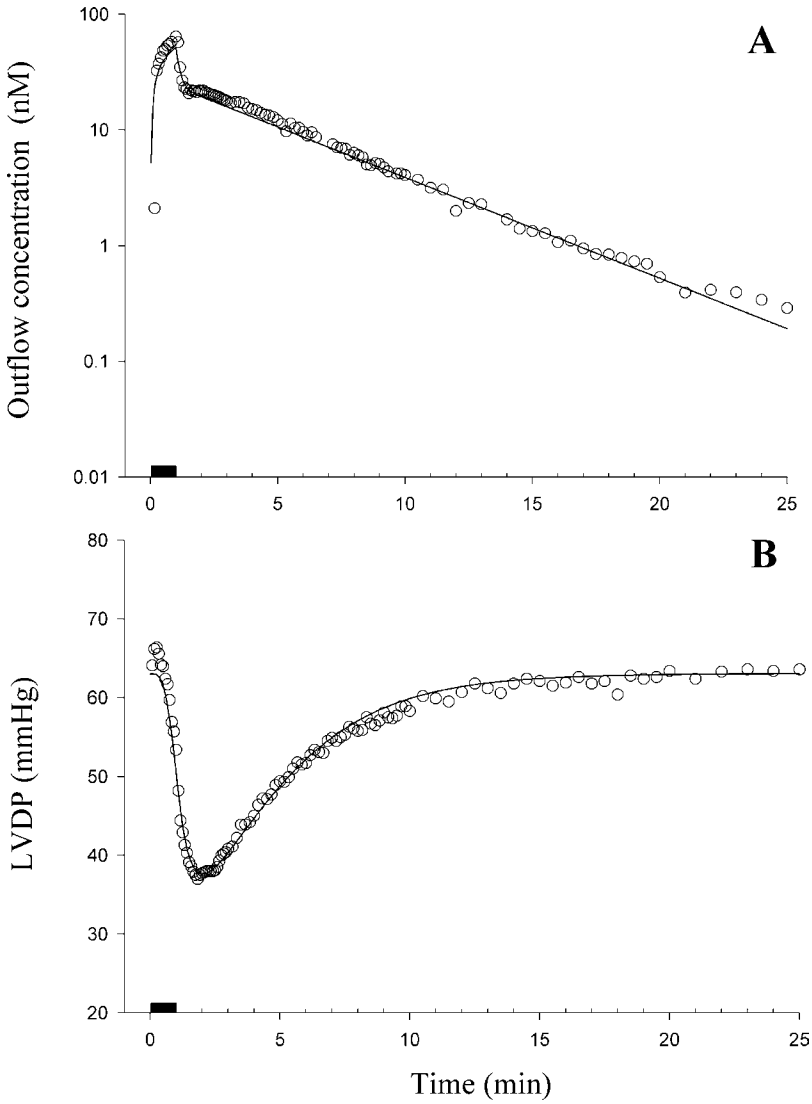

Fig. 5. (A) Representative fits of outflow concentration and (B) negative inotropic effect in one heart for a 1-min infusion 1.5 nmol of verapamil in the presence of amiodarone (1 μM) in perfusate.

neutral lipids and phospholipids as well as binding to acidic phospholipids into account (24). Although it appears hardly possible to identify uniquely different classes of binding sites on the basis of these data, one may speculate that slow binding (k_{ir} = 0.04 min^{-1}) is due to electrostatic interaction with tissue acidic phospholipids (25). That we found an apparent irreversible myocardial binding may be due to the experimental design, i.e., the limited observation time determined by the sensitivity of the analytical method; a model with reversible binding was not identifiable. In principle, cardiac metabolism of verapamil could be considered as an alternative explanation (26,27); however, no data on the resulting extraction of verapamil in the perfused rat heart are yet available and extrapolating the *in vitro* data, one would expect a value of less than 1%.

It appears most interesting, however, that the Pgp inhibitor amiodarone neither increased the cardiac uptake rate nor the equilibrium partition coefficient of the Pgp substrate verapamil. In the presence of amiodarone at the same concentration, cardiac uptake of idarubicin, in contrast, nearly doubled (12). In the light of the results obtained here for verapamil, it is open for discussion whether this increase in idarubicin uptake can be attributed to Pgp inhibition. Alternative explanations that could account for the increase in myocardial uptake of idarubicin in the presence of Pgp inhibitors have been based on drug–lipid interactions (13), e.g., an increase in membrane fluidity that leads to increased membrane permeability (28). Recently, it was shown that idarubicin is transported across membranes by a fast flip-flop process that is less influenced by Pgp (14). Note further that in contrast to verapamil, the cardiac uptake of idarubicin was saturable and could be inhibited by doxorubicin (12,29). This unexpected lack of an increased cardiac uptake of the Pgp substrate verapamil in the presence of amiodarone puts new questions to the functional consequences of cardiac Pgp expression (7).

Table I. Parameter Estimates (SD in Parenthesis) from Fitting of Verapamil Outflow and Negative Inotropic Response Data for a 1-min Infusion 1.5 nmol of Verapamil in Rat Hearts under Control Conditions and in the Presence of 1 μM Amiodarone

Parameter	Control single dose (n = 8) mean (SD)	Control double dose (n = 8) mean (SD)	Amiodarone single dose (n = 4) mean (SD)
Pharmacokinetics			
k_{in}(min^{-1})	635.0 (184.0)	679.0 (167.0)	587.0 (197.4)
CV[a](%)	6.4 (1.3)	4.6 (0.7)	7.6 (2.8)
k_{out}(min^{-1})	0.740 (0.078)	0.739 (0.187)	0.865 (0.162)
CV(%)	5.2 (1.0)	3.9 (0.8)	6.3 (2.0)
k_{ir}(min^{-1})	0.035 (0.029)	0.035 (0.027)	0.024 (0.029)
CV(%)	13.6 (11.5)	4.9 (4.1)	8.9 (7.1)
V_{ss}(ml)	51.2 (11.6)	56.4 (12.6)	40.3 (8.8)
CV(%)	2.8 (0.6)	2.3 (0.5)	3.3 (1.2)
Pharmacodynamics			
E_{max}(mmHg)	50.5 (18.9)	42.7 (18.3)	38.8 (23.0)
CV(%)	13.1 (6.6)	28.5 (26.32)	48.4 (11.8)
EC_{50}(nM)	16.4 (4.1)	12.8 (6.9)	21.1 (7.7)
CV(%)	12.9 (8.3)	29.7 (40.8)	32.6 (42.3)
N	2.1 (0.6)	2.3 (1.2)	2.3 (0.7)
CV(%)	8.6 (2.1)	11.9 (7.4)	9.6 (9.1)
τ_e(min)	0.34 (0.15)	0.36 (0.10)	0.46 (0.05)
CV(%)	8.8 (12.5)	6.3 (4.3)	4.2 (0.03)
g	0.27 (0.22)	0.16 (0.11)	0.10 (0.09)
CV(%)	60.4 (67.2)	6.7 (5.0)	369 (434)
τ_m(min)	6.25 (4.86)	2.98 (1.25)	16.9* (13.2)
CV(%)	81.0 (171.3)	8.0 (7.7)	165 (169)
E_0(mmHg)	71.1 (18.6)	71.6 (15.8)	65.8 (4.9)
CV(%)	0.7 (0.2)	0.7 (0.5)	0.8 (0.1)

The results obtained in double-dose experiments (0.75 nmol Verapamil at t = 0 and 10 min) under control conditions are also shown.
[a] Asymptotic CV(%) as measure of imprecision of individual parameter estimates.
*$P < 0.05$ *vs* control (double-dose).

Pharmacodynamics

The time course of the negative inotropic effect and positive inotropic 'rebound' upon cessation of 1-min verapamil infusion is quite well described by our model (Fig. 3B). The validity of the tolerance model is further supported by its usefulness in fitting the response to double dosing, where half of the single dose was administered at t = 0 and 10 min (Fig. 4B) and the fact that the PD parameters estimated in these hearts were similar (not statistically different) from those obtained in the single dose group. As expected, the parameters describing the delay and extent of tolerance development (τ_m, g) were estimated with much less precision in single dose experiments, where the observation time was shorter relative to MTT_{tol}, than in double dose experiments. Thus, we think that a mean delay time of 12 min and a g value of 0.16, corresponding to a 16% reduction of the negative inotropic effect at steady-state, represent the relevant parameters of tolerance development. However, the possibility of a dependency of tolerance development on drug input rate should be also taken into account. The high SD of τ_m and g estimates (CV in the order of 80%) suggests that there is a high interindividual variability in tolerance development. Note that it can be excluded that the observed rebound phenomenon is caused by a baseline shift since in separate experiments no significant change in baseline value of LVDP could be detected over an observation time of 40 min (n = 5, data not shown) and, if at all, then such a shift would be expected in the opposite direction. In developing the model, we tested all relevant models of acute tolerance reviewed by Gardmark *et al.* (30), however, non of these models could predict both the rebound effect and the steady-state behavior of the system. The present approach is the most parsimonious model for tolerance and rebound phenomena that could be found for verapamil. With respect to the compensatory feedback mechanism, our model is similar to the "direct moderator model" (18,30) characterized by an additive reduction of the effect. However, as Gardmark *et al.* (30) pointed out, all available tolerance models are empirical since, first, a mechanistic model would be too complex and, second, the underlying mechanisms are unknown. This also applies here but the following possibilities could be considered, for example: a compensatory feedback release of endothelium-derived substances (31) or counter-regulation at the level of cellular Ca^{2+} transport, e.g., the participation of reverse mode Na^+/Ca^{2+} exchanger. In view of the latter, it may be interesting to note that amiodarone inhibits the Na^+/Ca^{2+} exchanger (32). That to our knowledge such a rebound effect was not previously reported for verapamil is not surprising in view of the fact that only apparent steady-state data (after stepwise infusion) but no single dose response data in the perfused heart were published so far (33,34).

The EC_{50} of 16.4 nM (verapamil concentration causing half the maximum negative inotropic response) estimated from the response to 1-min verapamil infusion of 1.5 nmol using PK/PD modeling was lower than that of 31.6 nM estimated in the traditional way from cumulative drug

infusion experiments. Using the latter method in rat hearts, values of 50 nM and 79 nM were reported by Hess *et al.* (33) and Kolar *et al.* (34). Since there is no proof that steady-state was attained in these stepwise infusion experiments, these estimates should be regarded with caution. It is important to note, however, that the response data observed in our stepwise infusion experiments (mean values) are in reasonable agreement with those simulated with the model (using the mean parameter estimates of the double dose experiment). Assuming plasma protein binding of 90%, the EC_{50} determined in sheep for (−) verapamil by Huang *et al.* (5) using single dose experiments corresponds to a free concentration of 12 nM, which is in reasonable agreement with our estimate. Note that the human therapeutic concentration of 41 nM free drug is in the order of the EC_{25} of the negative inotropic effect of verapamil measured in isolated human papillary muscle strips (35). Verapamil suppresses L-type Ca^{2+} channels (and myocardial contractility) by binding at a site accessible from the inside of the cell (36). Thus, the temporal delay between negative inotropic effect and unbound concentration in cardiac tissue, characterized by the time constant τ_D of 19 s, could be caused by membrane transport, binding to receptors and the cellular effectuation process. Interestingly, a time constant τ_{step} of ~10 s and an equilibrium dissociation constant K_d of 58 nM were obtained from the kinetic constants of verapamil binding to cardiac membrane vesicles (37). The kinetics of block by verapamil of L-type calcium current in rat myocytes led to a time constant of the same order of magnitude (38). Note that under *in vivo* conditions, the arterial input concentration profile changes slowly relative to the step input in the present case, which leads to a loss of kinetic information on cardiac uptake rate of verapamil (4,5). Note further that the mean transit time, V_{ss}/Q (~5 min), is the counterpart of the equilibration time ($1/k_{eo}$) between arterial and effect site concentration estimated *in vivo* using the traditional link model. Our estimate is in reasonable agreement with the estimates obtained *in vivo* (5). As expected for flow limited myocardial equilibration, the delay due to other transport and effectuation processes is negligible.

In the presence of amiodarone, only the maximum negative inotropic effect was significantly reduced if expressed as percent change from initial (pre-drug) values (Fig. 2). Because of the negative inotropic action of amiodarone (17) a baseline reduction was expected. However, the intra-individual difference of 14% was not significant, and as expected this change is even less obvious in comparing the groups, where a non-significant (~8%) reduction of E_0 was observed. An additivity of the negative inotropic effects of amiodarone and verapamil (39) would be in accordance with the fact that the negative inotropic effect of amiodarone is mainly mediated by its calcium channel blocking action (40). The small reduction in CVR by verapamil in the presence of amiodarone (only 4% decrease) may be due to limitation to maximal response since amiodarone already reduced the baseline of CVR by 42%. This coronary vasodilating effect of amiodarone is well established and recently shown to be mediated primarily by the nitric oxide pathway (41).

In summary, the present results suggest that cardiac uptake of verapamil is rapid and unaffected by amiodarone. A PK/PD model that accounts for acute tolerance has been successfully applied to the negative inotropic verapamil effect. Amiodarone tended to suppress tolerance development. It will be important to further investigate the effects of Pgp inhibition on cardiac uptake of Pgp substrates in view of a potential role in drug–drug interactions (7,42).

ACKNOWLEDGMENTS

We thank the reviewers for insightful comments. Pakawadee Sermsappasuk is supported by a Royal Thai Government scholarship under the Committee Staff Development Project of Commission on Higher Education.

REFERENCES

1. W. A. Catterall and J. Striessnig. Receptor sites for Ca^{2+} channel antagonists. *Trends Pharmacol. Sci.* **13**:256–262 (1992).
2. I. Bodi, G. Mikala, S. E. Koch, S. A. Akhter, and A. Schwartz. The L-type calcium channel in the heart: the beat goes on. *J. Clin. Invest.* **115**:3306–3317 (2005).
3. M. J. Eisenberg, A. Brox, and A. N. Bestawros. Calcium channel blockers: an update. *Am. J. Med.* **116**:35–43 (2004).
4. A. C. Powell, J. D. Horowitz, P. J. Kertes, Y. Hasin, M. L. Syrjanen, C. A. Henry, D. M. Sartor, and W. J. Louis. Determinants of acute hemodynamic and electrophysiologic effects of verapamil in humans: role of myocardial drug uptake. *J. Cardiovasc. Pharmacol.* **16**:572–583 (1990).
5. Y. F. Huang, R. N. Upton, D. Zheng, C. McLean, E. C. Gray, and C. Grant. The enantiomer-specific kinetics and dynamics of verapamil after rapid intravenous administration to sheep: physiological analysis and modeling. *J. Pharmacol. Exp. Ther.* **284**:1048–1057 (1998).
6. L. Sasongko, J. M. Link, M. Muzi, D. A. Mankoff, X. Yang, A. C. Collier, S. C. Shoner, and J. D. Unadkat. Imaging P-glycoprotein transport activity at the human blood-brain barrier with positron emission tomography. *Clin. Pharmacol. Ther.* **77**:503–514 (2005).
7. L. Couture, J. A. Nash, and J. Turgeon. The ATP-binding cassette transporters and their implication in drug disposition: a special look at the heart. *Pharmacol. Rev.* **58**:244–258 (2006).
8. K. Meissner, B. Sperker, C. Karsten, H. M. Zu Schwabedissen, U. Seeland, M. Bohm, S. Bien, P. Dazert, C. Kunert-Keil, S. Vogelgesang, R. Warzok, W. Siegmund, I. Cascorbi, M. Wendt, and H. K. Kroemer. Expression and localization of P-glycoprotein in human heart: effects of cardiomyopathy. *J. Histochem. Cytochem.* **50**:1351–1356 (2002).
9. A. J. Lazarowski, H. J. Garcia Rivello, G. L. Vera Janavel, L. A. Cuniberti, P. M. Cabeza Meckert, G. G. Yannarelli, A. Mele, A. J. Crottogini, and R. P. Laguens. Cardiomyocytes of chronically ischemic pig hearts express the MDR-1 gene-encoded P-glycoprotein. *J. Histochem. Cytochem.* **53**:845–850 (2005).
10. J. H. Wang, D. A. Scollard, S. Teng, R. M. Reilly, and M. Piquette-Miller. Detection of P-glycoprotein activity in endotoxemic rats by 99mTc-sestamibi imaging. *J. Nucl. Med.* **46**:1537–1545 (2005).
11. J. van Asperen, O. van Tellingen, F. Tijssen, A. H. Schinkel, and J. H. Beijnen. Increased accumulation of doxorubicin and doxorubicinol in cardiac tissue of mice lacking mdr1a P-glycoprotein. *Br. J. Cancer* **79**:108–113 (1999).
12. M. Weiss and W. Kang. P-glycoprotein inhibitors enhance saturable uptake of idarubicin in rat heart: pharmacokinetic/pharmacodynamic modeling. *J. Pharmacol. Exp. Ther.* **300**:688–694 (2002).
13. G. Speelmans, R. W. Staffhorst, F. A. De Wolf, and B. De Kruijff. Verapamil competes with doxorubicin for binding to anionic phospholipids resulting in increased internal concen-

trations and rates of passive transport of doxorubicin. *Biochim. Biophys. Acta* **1238**:137–146 (1995).
14. R. Regev, D. Yeheskely-Hayon, H. Katzir, and G. D. Eytan. Transport of anthracyclines and mitoxantrone across membranes by a flip-flop mechanism. *Biochem. Pharmacol.* **70**:161–169 (2005).
15. W. D. Stein. Kinetics of the multidrug transporter (P-glycoprotein) and its reversal. *Physiol. Rev.* **77**:545–590 (1997).
16. G. P. Dobson and J. H. Cieslar. Intracellular, interstitial and plasma spaces in the rat myocardium *in vivo*. *J. Mol. Cell Cardiol.* **29**:3357–3363 (1997).
17. P. Sermsappasuk, M. Baek, and M. Weiss. Kinetic analysis of myocardial uptake and negative inotropic effect of amiodarone in rat heart. *Eur. J. Pharm. Sci.* **28**:243–248 (2006).
18. J. W. Mandema and D. R. Wada. Pharmacodymic model for acute tolerance development to the electroencephalographic effects of alfentanil in the rat. *J. Pharmacol. Exp. Ther.* **275**:1185–1194 (1995).
19. Y. N. Sun and W. J. Jusko. Transit compartments *versus* gamma distribution function to model signal transduction processes in pharmacodynamics. *J. Pharm. Sci.* **87**:732–737 (1998).
20. D. Z. D'Argenio and A. Schumitzky. *ADAPT II User's guide: Pharmacokinetic/Pharmacodynamic Systems Analysis Software*, Biomedical Simulations Resource, Los Angeles, 1997.
21. M. Weiss, M. Baek, and W. Kang. Systems analysis of digoxin kinetics and inotropic response in rat heart: effects of calcium and KB-R7943. *Am. J. Physiol. Heart Circ. Physiol.* **287**:H1857–H1867 (2004).
22. D. L. Keefe, Y. G. Yee, and R. E. Kates. Verapamil protein binding in patients and in normal subjects. *Clin. Pharmacol. Ther.* **29**:21–26 (1981).
23. D. L. Keefe and R. E. Kates. Myocardial disposition and cardiac pharmacodynamics of verapamil in the dog. *J. Pharmacol. Exp. Ther.* **220**:91–96 (1982).
24. T. Rodgers, D. Leahy, and M. Rowland. Physiologically based pharmacokinetic modeling 1: predicting the tissue distribution of moderate-to-strong bases. *J. Pharm. Sci.* **94**:1259–1276 (2005).
25. R. P. Mason, G. E. Gonye, D. W. Chester, and L. G. Herbette. Partitioning and location of Bay K 8644, 1,4-dihydropyridine calcium channel agonist, in model and biological membranes. *Biophys. J.* **55**:769–778 (1989).
26. M. Walles, T. Thum, K. Levsen, and J. Borlak. Verapamil metabolism in distinct regions of the heart and in cultures of cardiomyocytes of adult rats. *Drug Metab. Dispos.* **29**:761–768 (2001).
27. J. Borlak, M. Walles, K. Levsen, and T. Thum. Verapamil: metabolism in cultures of primary human coronary arterial endothelial cells. *Drug Metab. Dispos.* **31**:888–891 (2003).
28. S. Drori, G. D. Eytan, and Y. G. Assaraf. Potentiation of anticancer-drug cytotoxicity by multidrug-resistance chemosensitizers involves alterations in membrane fluidity leading to increased membrane permeability. *Eur. J. Biochem.* **228**:1020–1029 (1995).
29. W. Kang and M. Weiss. Kinetic analysis of saturable myocardial uptake of idarubicin in rat heart. Effect of doxorubicin and hypothermia. *Pharm. Res.* **20**:58–63 (2003).
30. M. Gardmark, L. Brynne, M. Hammarlund-Udenaes, and M. O. Karlsson. Interchangeability and predictive performance of empirical tolerance models. *Clin. Pharmacokinet.* **36**:145–167 (1999).
31. D. L. Brutsaert. Cardiac endothelial–myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. *Physiol. Rev.* **83**:59–115 (2003).
32. Y. Watanabe and J. Kimura. Inhibitory effect of amiodarone on Na(+)/Ca(2+) exchange current in guinea-pig cardiac myocytes. *Br. J. Pharmacol.* **131**:80–84 (2000).
33. M. E. Hess, J. Shanfeld, and N. R. Levine. Metabolic and inotropic effects of verapamil in perfused rat heart. *Recent Adv. Stud. Cardiac Struct. Metab.* **10**:81–88 (1975).
34. F. Kolar, B. Ost'adal, and F. Papousek. Effect of verapamil on contractile function of the isolated perfused rat heart during postnatal ontogeny. *Basic Res. Cardiol.* **85**:429–434 (1990).
35. R. H. Schwinger, M. Bohm, and E. Erdmann. Negative inotropic properties of isradipine, nifedipine, diltiazem, and verapamil in diseased human myocardial tissue. *J. Cardiovasc. Pharmacol.* **15**:892–899 (1990).
36. G. H. Hockerman, B. Z. Peterson, B. D. Johnson, and W. A. Catterall. Molecular determinants of drug binding and action on L-type calcium channels. *Annu. Rev. Pharmacol. Toxicol.* **37**:361–396 (1997).
37. M. L. Garcia, M. J. Trumble, J. P. Reuben, and G. J. Kaczorowski. Characterization of verapamil binding sites in cardiac membrane vesicles. *J. Biol. Chem.* **259**:15013–15016 (1984).
38. H. Nawrath and J. W. Wegener. Kinetics and state-dependent effects of verapamil on cardiac L-type calcium channels. *Naunyn–Schmiedebergs Arch. Pharmacol.* **355**:79–86 (1997).
39. T. J. Campbell and K. M. Williams. Therapeutic drug monitoring: Antiarrhythmic drugs. *Br. J. Clin. Pharmacol.* **46**:307–319 (1998).
40. A. Sugiyama, Y. Satoh, and K. Hashimoto. Acute electropharmacological effects of intravenously administered amiodarone assessed in the *in vivo* canine model. *Jpn. J. Pharmacol.* **87**:74–82 (2001).
41. P. Guiraudou, S. C. Pucheu, R. Gayraud, P. Gautier, A. Roccon, J. M. Herbert, and D. Nisato. Involvement of nitric oxide in amiodarone- and dronedarone-induced coronary vasodilation in guinea pig heart. *Eur. J. Pharmacol.* **496**:119–127 (2004).
42. D. Balayssac, N. Authier, A. Cayre, and F. Coudore. Does inhibition of P-glycoprotein lead to drug–drug interactions? *Toxicol. Lett.* **156**:319–329 (2005).