Influence of P-Glycoprotein Modulators on Cardiac Uptake, Metabolism, and Effects of Idarubicin

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Received May 27, 2001; accepted August 3, 2001

Purpose. The clinical utility of anthracyclines like idarubicin (IDA) is limited by the occurrence of multidrug resistance and cardiotoxicity. Previous studies have demonstrated that the multidrug transporter P-glycoprotein (P-gp) is present in the heart and have suggested that it exerts a protective function. We sought to determine the influence of P-gp inhibitors verapamil and PSC 833 on myocardial uptake, metabolism, and actions of IDA.

Methods. In Langendorff-perfused rat hearts, the outflow concentration-time curve and the residual amount in cardiac tissue of IDA and its active metabolite idarubicinol (IDOL) were measured after 0.5 mg dose of IDA in the absence and presence of the P-gp inhibitors verapamil and PSC 833.

Results. During perfusion (80 min), 2% of the IDA dose was converted to IDOL in the heart. Myocardial uptake of IDA was significantly increased by verapamil but not by PSC 833, which increased the recovery of IDA and IDOL. IDA significantly decreased left ventricular developed pressure to approximately 40% and increased coronary vascular resistance to 140% of baseline level, respectively. The vasoconstrictive effect was markedly potentiated by PSC 833.

Conclusions. The enhancement of myocardial IDA uptake by verapamil could be due to a decrease in P-gp-mediated efflux. PSC 833 inhibits cardiac metabolism (non-IDOL pathways) and increases the acute cardiotoxicity of IDA.

KEY WORDS: idarubicin; heart; verapamil; PSC 833; P-glycoprotein.

INTRODUCTION

The anthracycline antibiotic idarubicin (IDA) is effective against a wide range of human neoplasms; however, the clinical usefulness of anthracyclines is limited by a well-described but incompletely understood cardiac toxicity (1). The treatment of cancer with anthracyclines is further complicated by the phenomenon of multidrug resistance (MDR), caused by the (over)expression of the *MDR1* gene, which encodes the P-glycoprotein (P-gp). P-gp acts as an active plasma membrane drug efflux pump, decreasing intracellular concentrations of cytotoxic drugs. However, P-gp expression is not only confined to tumor cells but also present in numerous normal tissues including the heart (2,3). Thus, the absence of P-gp in *mdr1a(–/–)* mice resulted in increased cardiac levels of doxorubicin and doxorubicinol (4), and a combination of doxorubicin with P-gp inhibitors also increased its accumulation in the heart (5). These results suggest that the current strategy to reverse MDR by P-gp inhibitors might be accompanied by an increase in anthracycline cardiotoxicity (4,6).

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It has been hypothesized that IDA is less cardiotoxic than doxorubicin (1) and that it could overcome MDR due to its high lipophilicity (7). However, little has been reported on the effect of P-gp inhibitors on the myocardial pharmacokinetics and pharmacodynamics of IDA. The formation of its pharmacologically active alcohol metabolite idarubicinol (IDOL) in the heart tissue is of special interest because it has been speculated that the cardiac toxicity of anthracyclines could be related to its myocardial metabolism (8). To examine the myocardial uptake kinetics and effects of IDA, we used the isolated perfused Langendorff heart. This approach has the advantage in that the pharmacokinetics and pharmacodynamics can be investigated at the organ level without systemic influences because most P-gp inhibitors significantly alter disposition kinetics of IDA (and the formed IDOL) in the body. Thus, the present study was designed to characterize the cardiac uptake kinetics of IDA, its metabolism to IDOL, and the pharmacodynamic response after a single dose of IDA in the absence and presence of P-gp inhibitors. Thus, a second series of experiments was performed with perfusate containing verapamil and PSC 833 (valspodar), a cyclosporine D derivative that is nonnephrotoxic and nonimmunosuppressive.

MATERIALS AND METHODS

Materials

Idarubicin and verapamil were purchased from Pharmacia & Upjohn (Erlangen, Germany) and Sigma (Deisenhofen, Germany), respectively. PSC 833 (valspodar) was kindly donated by Novartis Pharma (Nürnberg, Germany). Valspodar 1.0-mL ampules contain 50 mg of PSC 833 dissolved in 60% Cremophor EL. The drug was diluted in perfusate to a final concentration of 1 μM. All other chemicals and solvents were of the highest grade available.

Perfused Rat Heart

The investigation conforms with the *Guide for the care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Prior approval was obtained from the Animal Protection Body of the State of Sachsen-Anhalt, Germany. Male Sprague-Dawley rats, 300 – 350 g, were anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneally). After the onset of general anesthesia, heparin (500 IU) was injected into the tail vein, and a cannula was bound into the trachea for ventilation. The chest was opened by making a bilateral incision from the costal margins to clavicles then folding the wall upward and dividing it into two pieces along sternum. To prevent ischemia, an aortic cannula filled with perfusate was inserted rapidly into the aorta, and retrograde perfusion was initiated with an oxygenated Krebs-Henseleit buffer solution. 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 mmHg in a non-recirculating way by the Langendorff technique. Perfusion pressure was regulated by a perfusion pressure control module (Type 671) and measured by a P23Db transducer (Bentley Trantec, Irvine, CA) connected to the aortic infusion cannula. A latex balloon attached to one

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end of a steel catheter was placed in the left ventricle through mitral valve. The catheter was filled with a mixture of ethanol and water (50:50), and the other end was linked to a linear recorder (Graphatec, Linearcoder 8300) via a second P23Db transducer. The perfusate consisted of Krebs–Henseleit buffer solution, pH 7.4, containing NaCl (118 mM), KCl (4.7 mM), $CaCl_2$ (2.52 mM), $MgSO_4$ (1.66 mM), $NaHCO_3$ (24.88 mM), KH_2PO_4 (1.18 mM), Glucose (5.55 mM), and Na-Pyruvate (2.0 mM). It was slightly flowed inside of a chamber saturated with 95% O_2–5% CO_2 and maintained at 37 °C. The latex balloon inserted in the left ventricle was periodically dilated with the mixture to produce 5–6 mmHg of left ventricular enddiastolic pressure (LVEDP). After a 10-min stabilization period to find reasonable coronary flow, the system was changed to constant flow condition whereby the coronary flow of 9.5 ± 0.4 mL/min was the result of an adjustment to about 10% of the initial value (to prevent an increase of perfusion pressure).

Experimental Protocol

Hearts (n = 15) were allowed to equilibrate for 20 min with Krebs–Henseleit solution. After the stabilization, 0.5 mL of IDA solution (1 mg/mL) was infused for 1 min with an infusion device. Outflow samples were collected every 10 s for 3 min, every 30 s for the next 7 min, every 60 s for the next 10 min, and every 5 min for the next 60 min (total collection period 80 min). Studies were performed under control conditions (n = 5) or during perfusion with Krebs–Henseleit buffer containing either 1 nM verapamil (n = 5) or 1 μM PSC 833 (n = 5), where the same dose of IDA was infused 20 min after start of perfusion of P-gp modulators. The outflow samples and hearts were kept frozen at –20°C until analysis.

Although the IDA dose was selected to induce submaximal negative inotropic and/or vasoconstrictive effects, the doses of verapamil and PSC 833 were below the threshold values, which led to changes in the measured cardiovascular effects (see below).

Determination of Idarubicin and Idarubicinol

IDA and IDOL were measured in the outflow perfusate samples and tissue extracts as previously described in detail (9). The high-performance liquid chromatography from Merck consisted of a pump (L-6200A), an autosampler (AS-2000A), and a fluorescence detector (RF-551) from Shimadzu. Chromatographic separation was performed using a LiChrospher 100 RP-18 (5μm; length: 4 mm, Merck). The mobile phase consisted of water:acetonitrile:tetrahydrofuran: H_3PO_4:triethylamin (312:165:20:1:2, v/v) and was adjusted to pH 2.2 with 5 M of HCl. The filtered mobile phase was degassed for 10 min with sonicator (Supersonic, Branson 2210, Danbury, CT). Flow rate was 1 mL/min, the excitation and emission wavelengths for fluorescence detection were set at 485 and 542 nm, respectively.

Tissue Concentration of Idarubicin and Idarubicinol

All hearts were divided randomly into four pieces and weighed. Each piece was homogenized in 2 mL of Na_2HPO_4 (0.5 M, pH 8.2) on ice with a tissue homogenizer (Ultra-Turrax T 25, IKA®-Labortechnik). Homogenates, 300–400mg, were taken from each fraction. Daunorubicinol (100 μL, 10 μg/mL), an internal standard, and 1 mL of Na_2HPO_4 were added and mixed with the homogenate. IDA and IDOL were extracted with 7 mL of chloroform:2-propanol [9:1 (v/v)]. After vortexing for 1 min and centrifuging (10 min at 2000g), the organic layer was recovered, evaporated for dryness under vacuum at 40°C, and reconstituted in 2 mL of methanol. Recoveries of IDA and IDOL were higher than 85.6% and 83.7%, respectively. The coefficients of variation of assay were less than 8.9% for IDA and 10.5% for IDOL.

Pharmacokinetics

The time course of amount of IDA (or IDOL) recovered in outflow perfusate, $A_R(t)$, was calculated from the outflow concentration vs. time data, $C(t)$, and perfusate flow, Q, using a numerical integration method:

$$A_R(t) = Q\int_0^t C(\tau)d\tau \quad (1)$$

and the fraction of dose recovered at the end of experiment (t_{last} = 80 min) is obtained as $Recovery = F_R(t_{last}) = A_R(t_{last})/D$. The amount of IDA *not* recovered into perfusate as IDA, $A_{NR}(t)$, is calculated as a function of time to characterize the myocardial kinetics of IDA:

$$A_{NR}(t)_{[0\rightarrow 1\text{min}]} = (D/\tau)\, t - A_R(t) \quad (2)$$

$$A_{NR}(t)_{[1\rightarrow 80\text{min}]} = A_{NR}(1) - A_R(t) \quad (3)$$

because $A_{NR}(t)$ reflects the time course of the residual amount of IDA in the heart plus generated metabolites (note that τ = 1 min is the infusion time).

Pharmacodynamics

Coronary perfusion pressure and the left ventricular pressure were recorded on a computer that allowed continuous monitoring of heart rate, left ventricular systolic pressure (LVSP), LVEDP, and maximum and minimum values of rate of left ventricular pressure development ($LVdP/dt_{max}$ and $LVdP/dt_{min}$). Coronary flow was measured by liquid level controller. Left ventricular developed pressure is defined as LVDP = LVSP – LVEDP. Analogous to Equation 1, numerical integration was used to calculate the area under the effect curve (AUE), which acts as a measure of the overall effect of IDA in the present situation of bolus injection where the pharmacological effect changes with time:

$$AUE = \int_0^{t_n} [E(t) - E_0]dt \quad (4)$$

where E_0 is the basal value (before IDA administration) and t_n denotes the time of the last measurement.

Statistics

The results are expressed as means ± stanard error of the mean. ANOVA and Student Newman–Keuls test were used to evaluate differences between groups and the significance of changes in the time course of effects was tested by one-way repeated measurement ANOVA. P values of less than 0.05 were considered statistically significant.

RESULTS

Uptake Kinetics and Metabolism of IDA

Figure 1 shows the average outflow concentration-time profiles for IDA and IDOL obtained for a 1-min infusion of IDA. The concentration of the formed IDOL was lower than that of the parent compound by a factor of about 10^{-3}. After reaching their peaks at the end of infusion, both curves decayed rapidly within 10 s, followed by a very slow decline. The IDOL outflow curve, in contrast, first increased (within 10 min) and then gradually decreased. The average time courses of the cardiac amount of IDA (plus formed metabolites) are depicted in Figure 2. The recoveries of IDA and IDOL in the outflow perfusate (up to 80 min) were 53.9 ± 8.5 and 0.28 ± 0.14% of the dose, whereas 15.9 ± 1.71 and 1.68 ± 0.15% remained in the heart; leading to a total recovery (cumulative outflow plus amount in tissue) of 69.7 ± 8.6 and 1.95 ± 0.24%, respectively. Thus, approximately 2% of the IDA dose was metabolized to IDOL during the time course of the experiment, whereas about 30% of dose was converted in the heart to unidentified metabolites.

Effect of Verapamil and PSC 833 on Pharmacokinetics of IDA and IDOL

As shown in Figure 2, verapamil (1 nM), in contrast to PSC 833 (1 μM), significantly increased cardiac uptake of IDA. The outflow recovery was decreased by 34.1% and residual amount was increased by 45.1% whereas for PSC 833, no effect on residual amount could be detected, and the outflow recovery was significantly increased by 43.8% (Fig. 3). However, there was a significant effect of PSC 833, but not of verapamil, on the outflow curve of the formed IDOL: the prevention of the decline of the curve after the second peak (Fig. 1) increased the recovery by 203.6% and decreased the residual amount in the heart by 53.6% (Fig. 3).

Pharmacodynamic Response of IDA

IDA (0.5 mg) decreased myocardial contractility (LVDP, $LVdP/dt_{max}$), with maximum effects at the end of infusion. The LVDP and $LVdP/dt_{max}$ were decreased to 39.3% and 36.2% of baseline level, respectively, and recovered within 30 min (Fig. 4). IDA impaired the diastolic relaxation; LVEDP increased from 5.3 ± 1.3 to 11.9 ± 3.4 mmHg after 2.4 min (Fig. 5) and $LVdP/dt_{min}$ decreased to 34.7% of baseline level. The time course of the IDA-induced change in coronary vascular resistance (CVR) was biphasic: after an initial increase to 138.2% (reaching a peak value at 2 min), it decreased to a minimum of –7.3% at 10 min and then increased again to 127.3% of the basal value at 80 min (Fig. 6). All these pharmacodynamic effects of IDA were significant at $P < .05$ level by one-way RM ANOVAs.

Effect of Verapamil and PSC 833 on IDA Pharmacodynamics

The perfusate concentrations of verapamil (1 nM) and PSC 833 (1 μM) did not induce cardiovascular effects. Verapamil attenuated the negative inotropic effect of IDA (Fig. 4), and the area under the effect curve AUE_{LVDP} changed from –324.4 ± 9.8 to –221.0 ± 28.2 mmHg min ($P < 0.05$). PSC 833, in contrast, impaired the recovery of LVDP to baseline (AUE_{LVDP} = –1724.6 mmHg min, $P < 0.05$ compared to control). The vasoconstrictive effect of IDA was markedly potentiated by PSC 833; both the maximum effect and the area under the effect curve AUE_{CVR} were significantly increased 2- and 3-fold, respectively. Verapamil significantly enhanced the secondary increase in CVR after 80 min (Fig. 6).

DISCUSSION

Only 2% of the IDA-dose was converted to IDOL in heart tissue. A myocardial concentration ratio IDOL to IDA

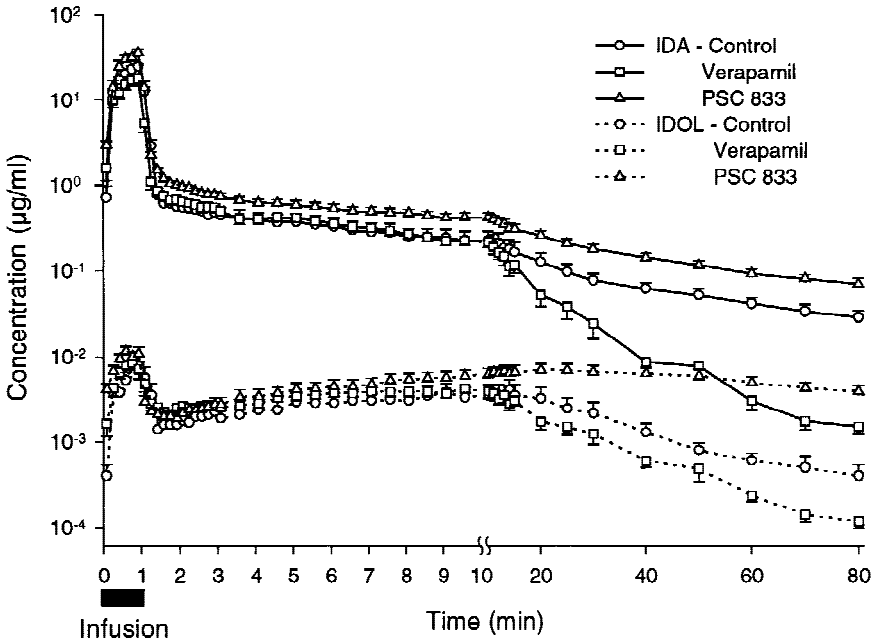

Fig. 1. Idarubicin (IDA) and idarubicinol (IDOL) outflow profiles in hearts perfused with Krebs–Henseleit solution (control), verapamil (1 nM), and PSC 833 (1 μM) after a 0.5-mg dose of IDA. Data points are the mean of five separate experiments ± standard error of the mean.

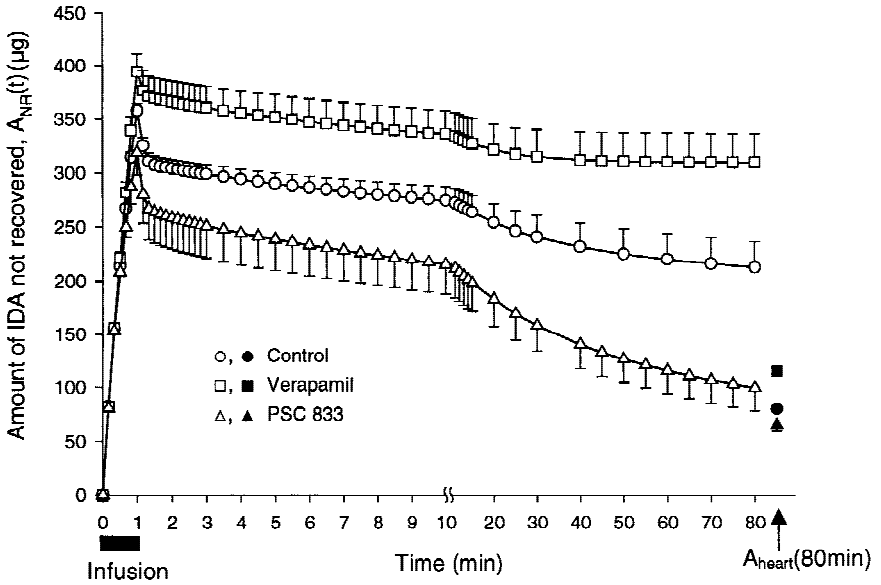

Fig. 2. Time courses of the idarubicin (IDA) amount not recovered up to time *t,* reflecting the amount of IDA plus formed metabolites in the heart. Data points are the mean of five separate experiments ± s.e.mean. The curves correspond to the outflow curves shown in Figure 1. A_{heart}(80 min) is the residual amount of IDA in the heart at the end of perfusion.

of 11% was observed at the end of the 80-min perfusion period after the single IDA dose of 0.5 mg whereas a value of 4% was measured by Platel *et al.* (10) in rat hearts after a 70-min perfusion with 2 nM IDA. The 13-hydroxy metabolite IDOL is formed primarily via carbonyl reduction of IDA (e.g., Reference 11), mainly in the liver but to a much less extent also in the heart as indicated by the results reported by Propper and Maser (12) for daunorubicin. Because the formation of nonpolar products, aglycones, represents a second major metabolic pathway of IDA metabolism (13), this route may account for the 30% of the dose not recovered as IDA. Thus, whereas the contribution of the generated IDOL to the time course of the amount not recovered $A_{NR}(t)$ is negligible, the contribution of other formed metabolites is substantial (Fig. 2).

This study shows that verapamil increases the uptake of IDA in the isolated perfused rat heart. This follows from the reduced recovery, i.e., the significant upward shift of the time course of the amount not recovered $A_{NR}(t)$ (Fig. 2) and the increase of the amount of IDA remaining in the heart at the end of perfusion. This finding could be explained by an inhibition of P-gp mediated transport as suggested by an increased accumulation of doxorubicin in mice lacking P-gp (5) and expression of P-gp in rat myocardium (14,15). Our data indicate that in the heart, P-gp-mediated tissue protection plays also a role for the highly lipophilic compound IDA. Furthermore, the inhibition of myocardial P-gp transport by the cardiovascular drug verapamil has clinical implications for predicting potential drug interactions (with P-gp substrates).

The expected effect of P-gp inhibition by PSC 833 on cardiac uptake of IDA was probably covered by other actions of PSC 833 on IDA disposition. The significant increase in

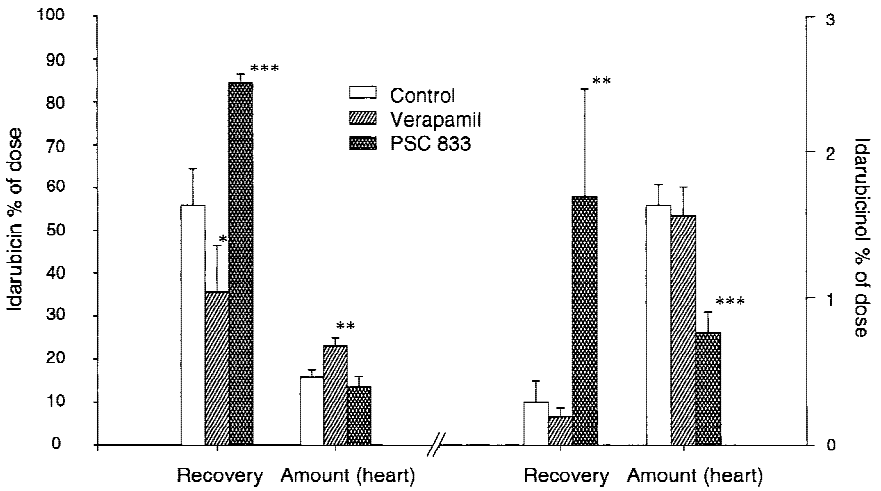

Fig. 3. Recovery of Idarubicin and idarubicinol (in perfusate and amount in heart at 80 min after infusion of idarubicin in hearts perfused with buffer (control), verapamil (1 nM), and PSC 833 (1 µM) (mean ± standard error of the mean; n = 5 in each group; *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$, compared to control).

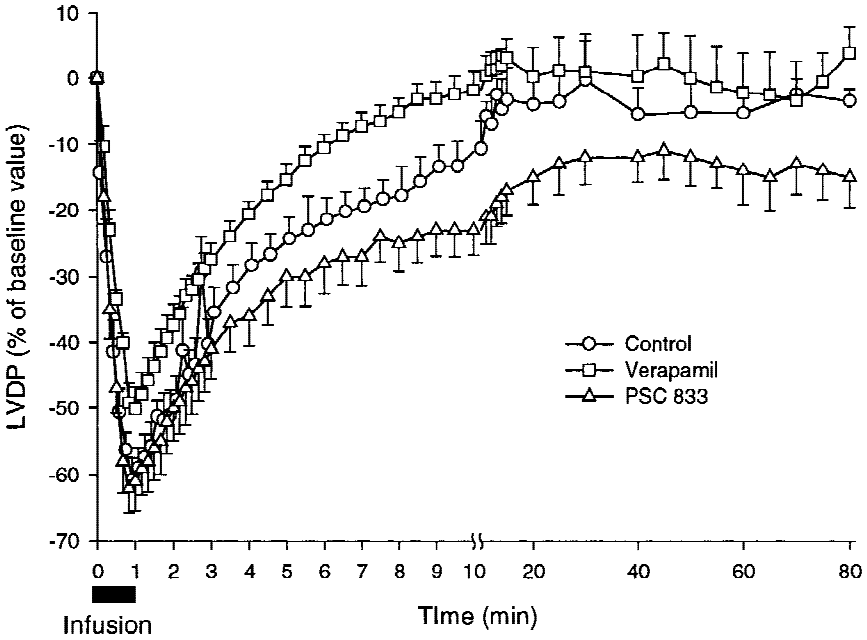

Fig. 4. Effect of idarubicin (0.5 mg) on time course of left ventricular developed pressure in hearts perfused with buffer (control), verapamil (1 nM), and PSC 833 (1 μM) (mean ± standard error of the mean; n = 5 in each group).

recovery of IDA and IDOL in the presence of PSC 833, i.e., the downward shift of the time course of the not recovered amount $A_{NR}(t)$ (Fig. 2) potentially relate to a decreased myocardial metabolism of IDA via other pathways than IDOL formation. Like other anthracyclines, IDA is also metabolized by microsomal glycosidase, which leads to aglycone formation, in addition to the measured IDOL formation by the cytoplasmic aldo-keto reductase pathway (13). Thus, PSC 833 may inhibit the conversion of IDA and IDOL to aglycone products; this suggestion is supported by the findings that 1) cyclosporine inhibits nitric oxide (NO) synthesis (see below) and 2) NO synthases may be responsible for the conversion of anthracyclines to aglycone products (16). Furthermore, because PSC 833 is also metabolized by cytochrome P450 reductase (17), a competitive inhibition could be taken into account. It should be noted that PSC 833 significantly increased the cardiac accumulation of doxorubicin administered intravenously to mice (5). On the other hand, Estevez *et al.* (15), who studied the interaction of P-gp modulators with doxorubicin in cultured rat cardiomyocytes observed a lower toxicity of doxorubicin in combination with PSC 833 than with verapamil.

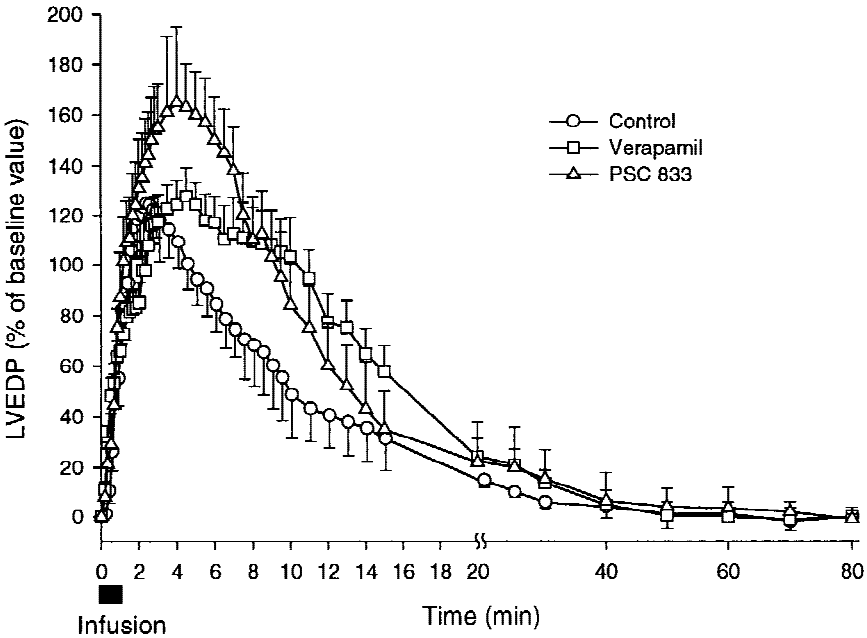

Fig. 5. Effect of idarubicin (0.5 mg) on left ventricular end diastolic pressure in hearts perfused with buffer (control), verapamil (1 nM), and PSC 833 (1 μM) (mean ± standard error of the mean; n = 5 in each group).

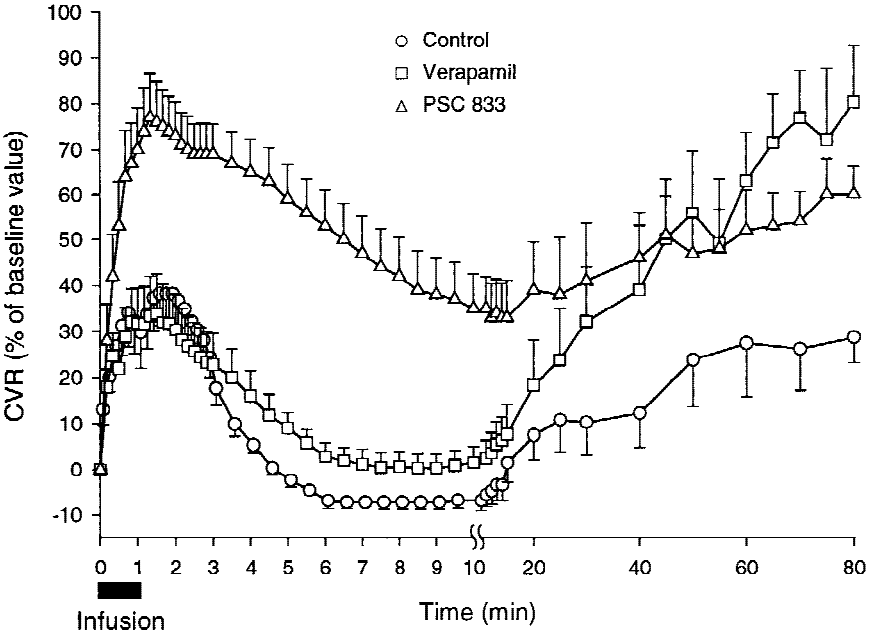

Fig. 6. Effect of idarubicin (0.5 mg) on coronary vascular resistance in hearts perfused with buffer (control), verapamil (1 nM), and PSC 833 (1 μM) (mean ± standard error of the mean; n = 5 in each group).

Acute time-, dose- and species-dependent cardiotoxicity characterized by negative inotropy and impairment of diastolic relaxation have been reported for anthracyclines, including IDA (1,10). Dysfunction of the sarcoplasmatic reticulum (leading to intracellular Ca^{2+} overload) and reactive oxygen radical production have been proposed as mechanisms involved (18). In view of the low fraction of IDOL generated from IDA, this toxicity is unlikely to be associated with the myocardial formation of the 13-dihydro metabolite as suggested for doxorubicin and daunorubicin (8,19). That verapamil significantly attenuated the IDA-induced decrease of LVDP is in accordance with results obtained for doxorubicin in the perfused rat heart (20) and recently reported protective effects of a calcium antagonist on doxorubicin-induced impairment of calcium transients in rat cardiac myocytes (21). Note that this pharmacodynamic effect of verapamil may be partly counterbalanced by its simultaneous enhancement of IDA uptake. (The latter may play a role for the increase in anthracycline cardiotoxicity upon chronic dosing; Reference 22.) PSC 833, in contrast, showed an opposite effect in prolonging the recovery of the IDA-induced LVDP decrease to baseline (Fig. 4). The same mechanisms that are responsible for the negative inotropic effects of cyclosporine A (23) might be involved in this interaction. There was also a tendency for an increase in LVEDP after coadministration of PSC 833, but the variability was too high to achieve statistical significance (Fig. 5).

It has been long recognized that the acute anthracycline cardiotoxicity is characterized by an increase in coronary resistance (24). Current knowledge suggests that inhibition of NO synthesis is most likely responsible for the vasoconstriction produced by IDA since there is no increasing evidence that 1) the release of NO contributes to the control of resting coronary blood flow (see e.g., References 2 and 25) anthracyclines are potent nitric oxide synthase (NOS) inhibitors as shown for doxorubicin (16), whereby the endothelial NO-synthase (NOS III) was particularly susceptible to this inhibition. Thus, acute NOS-inhibition increases coronary vascular resistance in Langendorff-perfused hearts (26). The potentiation of the IDA-induced vasoconstriction in the presence of PSC 833 could be explained by the same mechanism since it has been suggested that the acute vasoconstriction induced by cyclosporine may be (partly) mediated by an impaired NO release (27). Less is known regarding vascular effects of PSC 833; a calcium potentiating effect similar to that of cyclosporine was found in vascular smooth muscle cells (28). In a pilot experiment, we observed a dose-dependent increase in CVR with PSC 833 for concentrations above 0.1 mM (data not shown). Thus, while PSC 833 in the concentration used in the present study (1μM) did not affect CVR, it strongly potentiated the vasoconstrictive effect of IDA. The secondary increase in CVR in the presence of verapamil could be ascribed to the increase in the cardiac accumulation of IDA.

The importance of this study in our opinion is the measurement of both myocardial kinetics and dynamics of the Pg-p substrate IDA in the absence and presence of P-gp antagonists. It should be noted that while our verapamil perfusate concentration of 1nM is much lower than the concentrations used for MDR reversal *in vitro* and the therapeutic plasma concentrations, the concentration of PSC 833 (1 μM) is in the order of the effective concentration *in vitro* and *in vivo* (15,22). PSC 833 is clinically administered in an oil-based vehicle, Cremophor. We have made no attempt to differentiate between the effects of PSC 833 and Cremophor. Although Cremophor may also modulate the action of P-gp, it is at least 100 times less potent than PSC 833 (29). Furthermore, Cremophor has little impact, if any, on cardiac function in the applied concentration range (30).

In summary, we evaluated the effect of verapamil and PSC 833 on the pharmacokinetics and pharmacodynamics of IDA in the isolated perfused rat heart. The present study

indicates that while verapamil can increase the penetration of IDA in the rat heart, it attenuates the acute negative inotropic action of IDA. PSC 833, in contrast, enhanced the recovery of IDA and IDOL but potentiated the IDA-induced cardiotoxicity. These interactions at the organ level can be potentially complicating factors in therapeutic use of IDA when coadministered with verapamil or PSC 833.

ACKNOWLEDGMENTS

Part of this work was supported by Deutsche Forschungsgemeinschaft (GRK 134/1-96).

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