Kinetic Analysis of Saturable Myocardial Uptake of Idarubicin in Rat Heart: Effect of Doxorubicin and Hypothermia

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Purpose. Little is known of how anthracyclines are transported into the heart. Our previous kinetic study suggested saturable myocardial uptake of idarubicin. This study sought to determine the effects of temperature reduction and of doxorubicin coadministration on the transport process.

Methods. In Langendorff-perfused rat hearts, a 0.5 mg dose of idarubicin was infused over 10 min. The outflow concentration–time curve as well as the residual amounts in cardiac tissue of idarubicin and its active metabolite idarubicinol were measured after temperature reduction (from 37°C to 30°C) and in the presence of doxorubicin (20 μM) in the perfusate. The outflow concentration–time profile of idarubicin was analyzed by a four-compartment model and simultaneous nonlinear regression.

Results. Doxorubicin significantly inhibited the Michaelis–Menten-like uptake process of idarubicin in the heart (50% decrease in V_{max}), leading to a decreased net tissue uptake of idarubicin. Kinetic analysis of sensitivity to temperature reduction revealed a 2.6-fold increase in K_M; however, this inhibition of idarubicin uptake was counterbalanced by a decrease in efflux rate.

Conclusions. These data confirm the existence of a saturable myocardial uptake mechanism for idarubicin and might provide useful information for optimizing anthracycline dosage regiments.

KEY WORDS: idarubicin; heart; saturable uptake; hypothermia; doxorubicin.

INTRODUCTION

Idarubicin (IDA), a highly lipophilic anthracycline, is currently used in the treatment of acute leukemia and other malignancies. Cardiotoxicity has long been recognized as a complicating factor of cancer chemotherapy with anthracyclines. However, although the heart is a major target organ for anthracycline toxicity, there is limited quantitative knowledge about the uptake kinetics of anthracyclines into the myocardium. Previous uptake studies have been largely performed *in vitro* in tumor cell lines, and it is doubtful whether these results accurately reflect the transport in intact organs. Furthermore, controversy exists as to whether cellular uptake of anthracyclines occurs via saturable transport mechanisms or passive diffusion (e.g., Refs. 1–3).

The results of previous work performed in our laboratory using a rat Langendorff heart preparation have suggested that myocardial uptake of IDA appears to be characterized by Michaelis–Menten-type kinetics and that the negative inotropic effect of IDA is closely related to its cellular pharmacokinetics (4). However, the nature of this saturable transport process is unknown. Because IDA is a hydrophobic molecule, it would be expected to cross the membrane barriers passively with relative ease in neutral form (3), but the mechanism by which anthracyclines are transported across cell membranes in cultured cells still remains rather unclear. To our knowledge, only our previous report quantifies anthracycline uptake in an intact organ. Thus, the purpose of the current study was to investigate temperature dependence and inhibitability by doxorubicin of myocardial IDA uptake in order to further elucidate the underlying mechanisms. Pharmacokinetic modeling is a useful tool for analyzing drug disposition in isolated perfused organs. Here we used a similar experimental design and mathematical model as described previously (4). In addition to the analysis of IDA kinetics, the amount of active metabolite idarubicinol (IDOL) generated by the heart tissue was estimated.

One challenging task in improving anthracycline therapy is the determination of optimal dosage schedules (5). Studies in isolated perfused hearts may provide useful information for dosage optimization, not only because of cardiotoxicity but also because organ uptake in general is an important determinant for whole-body distribution kinetics of drugs (6). Data on doxorubicin disposition in rats demonstrated that the time course of cardiac tissue concentration is not correlated with plasma concentration and also indicated that cardiac uptake is a main determinant of cardiotoxicity (7).

MATERIALS AND METHODS

Materials

Idarubicin was purchased from Pharmacia-Upjohn (Erlangen, Germany). Doxorubicin was kindly donated by Norvatis Pharma (Nürnberg, Germany). All other chemicals and solvents were of the highest grade available.

Experimental Protocol

Hearts were isolated and perfused with standard procedures as described previously (8). The heart was isolated from male Sprague–Dawley rats (300–350 g), and retrograde perfusion was performed with an oxygenated Krebs–Henseleit buffer at 37°C in a single-pass way by the Langendorff technique. Coronary perfusion was initiated through a short cannula in the aortic root and maintained at a constant pressure of 60 mmHg, and a constant coronary flow of 9.5 ± 0.2 ml/min was maintained by a roller pump. A latex balloon was placed in the left ventricle of the isovolumetrically contracting heart and connected to a pressure transducer line (diastolic pressure was set to 5 to 6 mmHg). 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 physiologic recording system (Hugo Sachs Elektronik, March, Germany) was used to monitor left ventricular systolic (LVSP) and left ventricular end-diastolic pressure (LVEDP). Left ventricular developed pressure was calculated as LVDP = LVSP – LVEDP. After 20-min periods of equilibration and either infusion of doxorubicin (20 μM) or cooling from 37°C to 30°C, IDA (0.5 mg) was infused for 10 min with an infusion device into the

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ABBREVIATIONS: IDA, idarubicin; IDOL, idarubicinol; LVDP, left ventricular developed pressure; SNLR, simultaneous nonlinear regression.

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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 and hearts were assayed for IDA and its active metabolite IDOL by high-performance liquid chromatography (HPLC) as previously described (8). To evaluate the pharmacodynamic effects produced by doxorubicin alone, doxorubicin (20 μM) was infused for 100 min following a 20-min stabilization period. In each group independent experiments were performed in five hearts. The investigation conforms with the "Principles of Laboratory Animal Care" published by the US National Institutes of Health (NIH Publication 85-23, revised in 1985).

Model and Data Analysis

The pharmacokinetic modeling methodology has been described in detail previously (4). As shown in Fig. 1, perfusate flow (and drug input) as well as drug outflow occur in compartment 1 (distribution volume V_1), which reflects the initial distribution of IDA in the heart, probably because extracellular distribution and binding are fast relative to the infusion rate. Transport out of compartment 1 (cellular uptake) was saturable and described by a Michaelis–Menten-type process

$$J_{12}(t) = \frac{V_{\max,12} x_1(t)}{K_{M,12} + x_1(t)} \quad (1)$$

with apparent maximal transport rate $V_{\max,12}$ and apparent Michaelis constant $K_{M,12}$. First-order rate constants describing passive intercompartmental transport are denoted by k_{ij}. Compartments 2 and 3 may represent the cytosolic region and intracellular organelles or reversible binding sites in the intracellular space, respectively. k_{24} accounts for both metabolism and irreversible binding of IDA.

Because of the relatively large number of parameters, it would be difficult to obtain a unique solution (parameter estimates) fitting only one data set. Thus, it is an advantage of the present experimental design that the analysis can be based on data from different but related experiments, whose modeling functions share most of the parameters except those that account for the treatment effects. This method is called simultaneous nonlinear regression (SNLR). SNLR was performed using three data sets, the average data of the control group (4) and those of the doxorubicin and hypothermia groups. The parameters (Fig. 1) described the kinetics in the control group, and factors f_i accounted for potential changes in parameters P_i that resulted from the treatments (i.e., in the model of the treatment groups, model parameters P_i were replaced by f_i P_i). All possible combinations of factors were tested with the aim of describing treatment groups by a minimum number of factors, i.e., free model parameters. The model selection was made according to the following criteria. Any model showing a noninvertible Fisher's information matrix was discarded as nonidentifiable (9). Of the identifiable models, preference was given to those with low values of the Akaike information criterion (AIC) and to those whose pattern of residuals better approximated a random scatter. Differential equations describing the mass transport among compartments (Fig. 1) were solved numerically and fitted to the data using ADAPT II (10). SNLR was performed with average data values (with five hearts in each group) using maximum likelihood estimation and assuming that the measurement error has a standard deviation that is a linear function of the measured quantity. Parameter uncertainties and independence among parameters were verified using fractional standard deviations (*FSD*) and correlations obtained from the fitting procedure (11). We considered an $FSD > 0.5$ to indicate that a parameter was not estimated with sufficient statistical certainty.

The fraction of IDA dose recovered in the outflow perfusate at the end of the experiment [$A_R(t_{\text{last}})/Dose$, $t_{\text{last}} = 80$ min] was calculated model-independently from the outflow concentration vs. time data, $C(t)$, and perfusate flow, Q, using a numerical integration method as

$$A_R(t_{last}) = Q\int_0^{t_{last}} C(t)dt$$

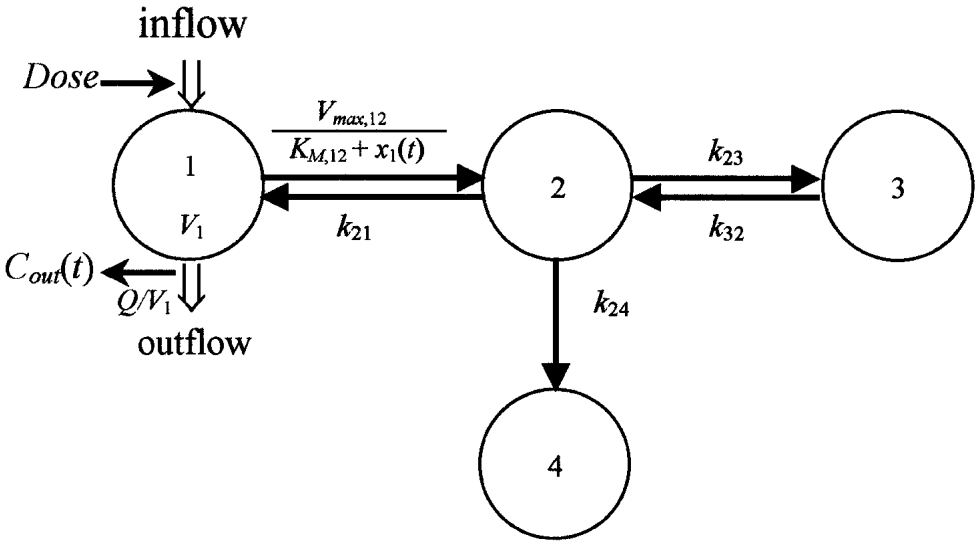

Fig. 1. Compartmental model of IDA kinetics in the isolated perfused rat heart (V_1, initial distribution volume; Q , perfusate flow; Q/V_1, outflow rate constant; k_{ij}, first-order rate constants; $V_{\max,12}$ and $K_{M,12}$, Michaelis–Menten parameters of the uptake transport process). Compartment 1 might account for the extracellular distribution of IDA, 2–4 represent cellular compartments.

Statistics

The data are presented as average ± S.D. The significance of changes in the time course of outflow concentration in the presence of doxorubicin was tested by one-way repeated-measurement ANOVA; p values less than 0.05 were considered statistically significant. The likelihood ratio test (12) was used to determine the significance of parameter changes in the nested models due to the treatments.

RESULTS

The averaged outflow concentration–time curves after infusion of 0.5 mg IDA for 10 min are shown in Fig. 2A for the control and two treatment groups ($n = 5$ independent experiments in each group). The curves showed a maximum at the end of infusion, and a rapid decay on cessation of infusion was followed by a slower terminal phase. In the presence of doxorubicin (20 μM), the curve was shifted upward in a nearly parallel fashion ($p < 0.01$), suggesting reduced uptake during infusion. Mild hypothermia (30°C) changed the shape of the outflow, $C(t)$, curve: following a steep increase within about 1 min (leaving the maximum at the end of infusion nearly unchanged), the concentration in the terminal phase was lower than that of control. Doxorubicin significantly inhibited net cardiac uptake of IDA as reflected by an increase in outflow recovery and a decrease in residual amount in the heart (Fig. 2D).

Figures 2B and 2C show the fits of outflow concentration–time profiles of the doxorubicin and hypothermia groups, respectively, obtained by SNLR of the pooled control and treatment data. The parameter estimates are summarized in Table I. Doxorubicin reduced both the maximal uptake rate $V_{max,12}$ and the cellular sequestration rate constant k_{24} (accounting for irreversible binding and metabolism) significantly to 49% and 37%, respectively, of the control values ($p < 0.01$). Hypothermia (30°C), in contrast, reduced IDA uptake rate by increasing the Michaelis constant $K_{M,12}$ (~2.6-fold greater than that for control). But because the efflux rate constant k_{21} also decreased (to 14% of the control value), the net uptake remained unchanged (Fig. 2D). The effect of temperature reduction on the model parameters $K_{M,12}$ and k_{21} was significant ($p < 0.01$). The presence of doxorubicin and hypothermia decreased the formation of idarubicinol from IDA in the heart by 85% ($p < 0.01$) and 34% ($p < 0.05$), respectively (Fig. 2D).

After an increase in LVDP from the positive inotropism

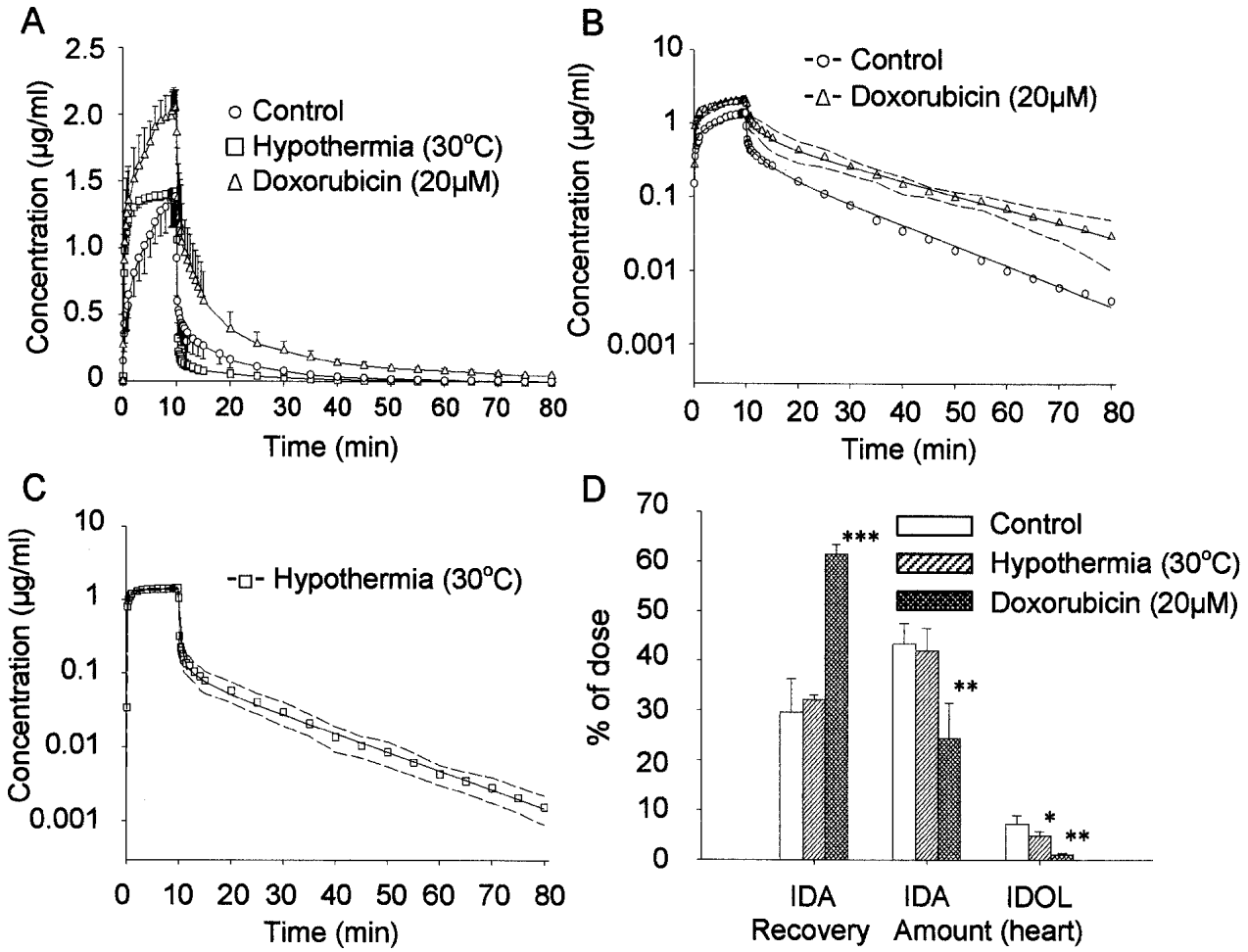

Fig. 2. A: IDA outflow profiles in hearts perfused with Krebs–Henseleit solution (control, 37°C), doxorubicin (20 μM), and hypothermia (30°C) for a 10-min infusion of 0.5 mg IDA (mean ± S.D., $n = 5$ in each group). B–C: Simultaneous fit of the mean outflow data of the control and doxorubicin (B) and low-temperature experiments (C). The *dashed lines* indicate 1 S.D. D: Outflow recoveries of IDA and residual amounts of IDA and IDOL in the heart at the end of experiment (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.005$ versus control).

Table I. Model Parameter Estimates[a] for the Disposition of IDA in Hearts from Control, Doxorubicin (20 μM) and Hypothermia (30°C) Group

Control	$V_{max,12}$ (nmol min^{-1})	$K_{M,12}$ (nmol)	k_{21} (min^{-1})	k_{23} (min^{-1})	k_{32} (min^{-1})	k_{24} (min^{-1})	V_1 (ml)
	974 (26)[b]	156 (29)	0.22 (17)	0.11 (38)	0.16 (10)	0.11 (14)	13.2 (3)
Doxorubicin[c]	$f_{Vmax,12,Dox}$ 0.49 (5)	—	—	—	—	$f_{k24,Dox}$ 0.37 (3)	—
Hypothermia[c]	—	$f_{KM,12,Hypo}$ 2.58 (6)	$f_{k21,Hypo}$ 0.14 (5)	—	—	—	—

[a] Simultaneous nonlinear regression of IDA outflow concentration-time profiles where two additional parameters accounted in the model equations for the effect of doxorubicin (20 μM) and hypothermia (30°C) on IDA.
[b] Fractional standard deviation of parameter estimates, FSD (%).
[c] Fractional change in parameter: e.g., $V_{max,12,Dox} = f_{Vmax,12,Dox} V_{max,12}$.

of hypothermia (to 160 ± 20% of baseline level), infusion of IDA decreased myocardial contractility (LVDP) to 37 ± 3% of preinfusion level. The effect recovered within 30 min to 62 ± 7% of preinfusion level (Fig. 3A). Although the presence of doxorubicin (20 μM) in the perfusate did not induce a significant change in LVDP within the first 20 min, infusion of IDA (0.5 mg) decreased LVDP to 49 ± 5% of its preinfusion value at the end of infusion, and recovery was completely impaired (after a transient increase to 64.4 ± 6% of preinfusion level) because of the delayed negative inotropic effect of doxorubicin. When doxorubicin was administered alone, its relatively low concentration (20 μM) produced a maximum decrease in LVDP (to 56.1 ± 9% of baseline level) about 100 min after start of infusion (Fig. 3B). All these pharmacodynamic effects of IDA were significant at the $p < 0.05$ level by one-way RM ANOVAs.

DISCUSSION

At present, there is very little mechanistic understanding of anthracycline transport into the heart and other organs.

In our first study on pharmacokinetic/pharmacodynamic modeling of idarubicin disposition in rat heart, we found a Michaelis–Menten-like uptake process; verapamil and amiodarone significantly increased idarubicin uptake (by an about twofold increase in V_{max}) (4). The main outcome of the current study was the inhibitability and temperature sensitivity of IDA uptake into the rat heart. The results indicate that our model adequately described the effects of doxorubicin and hypothermia on IDA uptake by the rat heart. The myocardial uptake rate of IDA was noncompetitively inhibited by another anthracycline, doxorubicin. (The inhibitory effect of doxorubicin was mediated through inhibition in the V_{max} of the IDA uptake process with no change in K_M.) On cooling from 37°C to 30°C an inhibition caused by an increase of the apparent Michaelis constant (K_M) was observed. Kinetic analysis of IDA data revealed that the significant decrease in maximum uptake rate V_{max} in the presence of doxorubicin led to a reduction of net uptake of IDA. The lower value of the sequestration rate constant k_{24}, which reflects a decrease in intracellular trapping and metabolism, is in line with the reduced metabolism to IDOL. Hypothermia, in contrast, did not affect net uptake of IDA because the inhibition of uptake rate (increase in K_M) was counterbalanced by a decrease in the efflux rate constant (k_{21}). Note that the factors $f_{Vmax12,Dox}$, $f_{k24,Dox}$ and $f_{KM12,Hypo}$, $f_{k21,Hypo}$ completely described the effect of doxorubicin and temperature, respectively, on pharmacokinetics of IDA since all data were simultaneously fitted by a single set of parameter values. Since the kinetic model (Fig. 1) was selected according to the principle

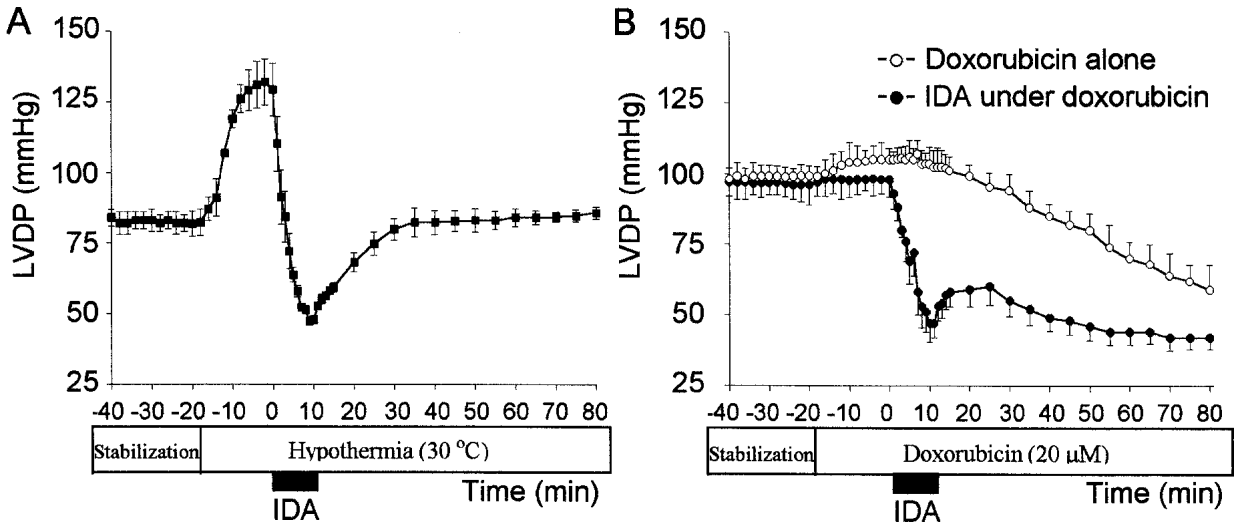

Fig. 3. Time course of left ventricular developed pressure (LVDP). A: Effect of mild hypothermia (cooling from 37°C to 30°C) and infusion of IDA (0.5 mg in 10 min). B: Effect of IDA in the presence of doxorubicin (20 μM) (●) and the effect of doxorubicin alone(○) (mean ± S.D., $n = 5$ in each group).

of parsimony as a minimal model, which is in accordance with the information content of the three outflow data sets, control, doxorubicin and hypothermia, the model structure and parameter estimates differ slightly from those reported previously (4). Thus, the second nonlinear transport process, which was necessary to link the concentration in compartment 2 with the time course of the negative inotropic effect of IDA, is now replaced by passive transport (k_{23}) because this led to a reduction of the AIC value from –743 to –756.

The noncompetitive inhibition of myocardial IDA uptake by doxorubicin is in accordance with observations in human leukemia HL60 cells and mononuclear cells (13). Interestingly, a competitive inhibition was observed for daunorubicin, suggesting that the latter, in contrast to doxorubicin, binds to the same site as IDA. A qualitatively similar effect of hypothermia was found for anthracycline uptake in cultured fibroblasts, where cellular accumulation of daunorubicin and doxorubicin was significantly inhibited by a decrease in incubation temperature from 37°C to 30°C (14). Hyperthermia, on the other hand, increased the cellular uptake of doxorubicin in cultured cells (15–17). The decrease in the efflux rate constant (k_{21}) with temperature is in line with observations in cultured cells, where the efflux rate of doxorubicin increased with temperature (16). Our findings support the suggestion that this hyperthermia-induced increase in cellular uptake cannot be explained by an influence on P-glycoprotein transport of doxorubicin (17).

It is well known that mild hypothermia exerts a positive inotropic effect (e.g., Ref. 18). The negative inotropic effect of IDA under hypothermia or doxorubicin was similar to that under control conditions (4). The complete impairment of LVDP recovery to preinfusion levels in the doxorubicin group is caused by the delayed development of the doxorubicin-induced negative inotropism. An explanation of this phenomenon is lacking, however, for the hypothermia group, where, similarly to the control experiments (4), LVDP recovered within 30 min but reached only 62% of the preinfusion level.

While the present results confirm the existence of a saturable myocardial uptake mechanism for idarubicin, the underlying mechanism remains unclear. Based on uptake studies in cultured kidney epithelial cells, Sasaya *et al.* (19) suggested that not the renal organic cation transporters (OCTs) but absorptive endocytosis might be involved in doxorubicin transport. Alternatively, it was hypothesized that the rate of drug transport may be dominated by partitioning of drug into the bilayer and saturation of nonspecific membrane binding sites could lead to a Michaelis–Menten-like transport process (2,19). Regev and Eytan (20) have shown that doxorubicin crosses membranes by a flip-flop mechanism. Although this nonmediated flip-flop movement is slow (time constant of about a minute), the flip-flop process may occur very rapidly (within milliseconds) when facilitating membrane proteins are involved (e.g., Ref. 21). The hypothesis that transport is dominated by partitioning of IDA into the membrane would be also in accordance with the observed temperature dependency (20,22). We have previously suggested that the apparent distribution volume of $V_1 = 14$ ml, which is orders of magnitude higher than the vascular space (~0.06 ml), reflects rapid partitioning into membranes (4). In other words, because of the slow input rate relative to this rapid equilibration process, the kinetics of the latter could not be resolved. This extremely rapid partitioning into the extracellular leaflet of the membrane is characteristic of amphipathic drugs (23,24). Thus, based on our minimal compartmental model, one cannot differentiate uniquely between binding and transport processes. Interestingly, cellular distribution kinetics of doxorubicin was also characterized by a fast and a slow process (25).

One aspect of pharmacokinetic studies on intact organs is their relevance for an interpretation of whole-body distribution kinetics. Saturability of tissue uptake may lead to a distribution kinetics that is dependent on the input rate (schedule of drug administration). This may provide a rationale for optimizing anthracycline dosage regimens, as recently suggested by El-Kareh and Secomb (5). Thus, it was hypothesized that dosage regimens leading to lower peak plasma concentrations of doxorubicin are associated with lower cardiotoxicity (26). Note that infusion of a 0.5-mg dose of IDA in 10 min (4) instead of 1 min (8) was accompanied by a reduced negative inotropic effect. Furthermore, possible differences in the uptake transport processes between normal and tumor tissues would offer a strategy for site-selective delivery of anthracyclines.

In summary, the results suggest that myocardial uptake of IDA in the rat heart is a temperature-dependent, doxorubicin-inhibitable, and saturable process. Further studies to characterize the underlying mechanism will be of importance.

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