Pharmaceutical Research, Vol. 6, No. 10, 1989

Research Article

Erythrocytes as Barriers for Drug Elimination in the Isolated Rat Liver. I. Doxorubicin

Hye-Jung Lee[1] and Win L. Chiou[1,2]

Received November 29, 1988; accepted April 14, 1989

The effect of doxorubicin (Dx) equilibration between plasma and erythrocytes (RBC), prior to entering the liver, on hepatic elimination was evaluated under two conditions: (I) the drug being first equilibrated for 30 min in the perfusate (containing 27% human RBC) before infusion into the liver and (II) the drug being directly infused into the liver. Mean ($N = 6$) steady-state hepatic extraction ratios (E) under conditions I and II were 0.286 ± 0.131 (SD) and 0.592 ± 0.147, respectively. The marked difference in E was attributed mainly to the initial difference in plasma/RBC Dx distribution ratio of the inlet blood, the slow efflux of Dx from RBC into plasma under condition I, and the slow influx of Dx from plasma to RBC under condition II. The results indicate that most Dx molecules in RBC are not available for elimination. Drug equilibration between plasma and RBC may therefore represent an important factor in hepatic first-pass metabolism.

KEY WORDS: erythrocyte barrier; hepatic elimination; isolated rat liver; doxorubicin; hepatic first-pass effect.

INTRODUCTION

Erythrocytes (RBC)[3] as a potential "barrier" for hepatic drug elimination from blood and its implications in pharmacokinetic studies (1–3) have not been fully studied to date. The fate of bound and/or unbound drug in plasma and in RBC during each hepatic transit has been assumed to be identical in most proposed hepatic models as well as in general pharmacokinetic theories (1), since the distribution equilibrium of a drug between plasma and RBC has been commonly assumed to be instantaneous. Upon review of the literature (2,4–7), Chiou (1) pointed out that for most drugs the influx from plasma into RBC and the efflux from RBC to plasma may be relatively slow compared to the mean hepatic transit time of blood [approximately 10 sec (1,2,8)]. Thus, any difference in the degree of equilibration of drugs between plasma and RBC may affect hepatic elimination. Further, the hepatic first-pass metabolism after oral administration may be greater than predicted from intravenous data, because the drug in blood entering the liver may be less well equilibrated (i.e., a greater fraction being present in blood plasma) after oral than after intravenous administration.

Doxorubicin (Dx) was employed as a model drug in the present study to test the above barrier effect. Differences in hepatic extraction and in plasma/RBC distribution were determined with the use of single-pass isolated perfused rat livers under two conditions: (I) the drug was infused after equilibration in "blood" and (II) the drug was infused directly without prior equilibration in blood. A preliminary evaluation regarding the Dx influx from plasma into RBC was also performed.

MATERIALS AND METHODS

Materials and Animals

Dx hydrochloride and doxorubicinol hydrochloride [a known metabolite (9)] were kindly donated by Adria Labs (Dublin, Ohio). All other chemicals were either reagent or HPLC grade (Fisher Scientific, Chicago). Filtered deionized water for the preparation of mobile phase, buffer, and normal saline was obtained from the Milli-Q water purification system (Millipore, Bedford, Mass.). Human RBC within 1 week after the expiration date was obtained from the blood bank of the University of Illinois Hospital. The blood cells outdated by more than 1 week were found to hemolyze easily during washing and perfusion experiments probably due to breakable membranes. The Dx stock solutions were pre-

[1] Department of Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612.

[2] To whom correspondence should be addressed at Department of Pharmacodynamics (M/C 865), College of Pharmacy, University of Illinois at Chicago, 833 South Wood Street, Chicago, Illinois 60612.

[3] Abbreviations used: RBC, erythrocytes; Dx, doxorubicin; TCA, trichloroacetic acid; E, extraction ratio; C_{in}, drug concentration in inlet blood; $C_{in,p}$, drug concentration in inlet plasma; $C_{in,r}$, drug concentration in inlet erythrocytes; C_{out}, drug concentration in outlet blood; $C_{out,p}$, drug concentration in outlet plasma; $C_{out,r}$, drug concentration in outlet erythrocytes; F_r, fraction of drug present in erythrocytes of blood; F_p, fraction of drug present in plasma of blood; B/P, blood-to-plasma drug concentration ratio; P/R, plasma-to-erythrocytes drug concentration ratio; E_p, fractional removal of drug from plasma of inlet blood; E_r, fractional removal of drug from erythrocytes of inlet blood; HPLC, high-performance liquid chromatography; HC, hematocrit.

0724-8741/89/1000-0833$06.00/0

pared by dissolving Dx in normal saline and adjusted to pH 7.4 with 1 *N* NaOH. These solutions were stable for 1 week at 4°C when protected from light (10). Male Sprague–Dawley albino rats (Bio-Lab, Oak Park, Ill.), weighing 300–420 g, were used as liver donors.

Liver Perfusion System

The single-pass isolated perfused rat liver system was prepared according to the method described by Pang (11) with slight modification. The left and right phrenic and vagus nerves, which are located along the jugular vein, were cut off to paralyze the diaphragm and to eliminate the possible secretion of neurotransmitters to the liver (12). The bile duct was not cannulated in the present study. The perfusion apparatus, equipped with two reservoir units (TWO/TEN Perfuser, MX International, Aurora, Colo.), was used. The perfusion medium containing about 27% washed RBC, 0.41% sucrose, and 0.3% glucose in Krebs–Ringer bicarbonate solution, was adjusted to pH 7.4 with 0.1 *N* NaOH. Heparin was added to the perfusion medium at a concentration of 5 U/ml before the perfusion started. The perfusion medium was maintained at 37°C and oxygenated with a humidified and warmed (13) 95% O_2–5% CO_2 mixture (Peterson Welding, Chicago) at a rate of 2 liters/min. The medium was kept at 4°C prior to the perfusion study and used on the day of preparation.

Perfusion of Doxorubicin

Dx was infused to six livers in a crossover fashion under two conditions (i.e., three livers were first studied under condition I followed by condition II, and the others in a reverse order). Under condition I, about 3–4 ml of a stock solution of Dx in normal saline (0.35 mg/ml) was added to the perfusion medium (about 450 ml) in the reservoir, and equilibration of the drug in "blood" (perfusion medium) and oxygenation were allowed for about 30 min prior to infusion. Under condition II, after similar oxygenation, the drug in Kreb's buffer (about 35 μg/ml; prepared from dilution of the stock solution) was directly infused at a rate of 1.1 ml/min through a 25-G needle into the portal vein catheter with the assistance of a Harvard infusion pump (Model 975, Southnatick, Mass.). In order to mix the drug solution more homogeneously in the portal vein, the end of the needle was blunted and covered with 1 cm of a silastic tubing. The tubing was sealed at the end with silicone (Medical Adhesive Silicone Type A, Dow Corning Co., Midland, Mich.) and punctured about 50 times with a 25-G needle. The drug solution used was previously filtered through a 0.22-μm filter paper. The Dx infusion rate was approximately 38 μg/min, and the perfusion flow was about 1.2 ml/min/g liver. The liver weight was estimated from the body weight (14). Before drug infusion under condition I or II, the liver was first infused with warmed and oxygenated blank perfusion medium for about 15 min.

Drug infusion period for conditions I and II was 25 min each. Inlet blood samples were collected at 0, 10, and 25 min from the reservoir for condition I and twice from the portal vein catheter before or at the end of the study for condition II. Two sets of outlet blood samples were collected into ice-cooled (used to minimize drug diffusion) microcentrifuge tubes every 5 min. One set was centrifuged immediately at 3000 rpm for 20 sec (Model 235A, Fisher Scientific) to obtain "plasma" samples. All plasma and blood samples were frozen using a dry ice–alcohol mixture and stored in a freezer (−20°C) until assay.

During the perfusion, the O_2 consumption of the inlet and outlet blood was determined periodically with an O_2 electrode (Model 97-08-00, Orion Research, Fisher Scientific) which was connected to a pH meter (Model 3500, Beckman Co., Fullerton, Calif.). Constant oxygen consumption was used to indicate an apparent stable liver preparation (15,16). A constant extraction ratio at steady state was also used to reflect a stable liver preparation (17).

Effect of the Storage Time of Outlet Blood Before Centrifugation on the Plasma/Erythrocytes Distribution of Doxorubicin

The effect of standing or storage of steady-state outlet blood samples (usually collected at 15 or 20 min after drug infusion) on plasma/RBC distribution of Dx was studied by measuring whole blood concentrations and concentrations in plasma from blood centrifuged after 0, 1, 3, 5, 10, and 15 min of standing at room temperature.

Influx Rate of Doxorubicin from Plasma into Erythrocytes

This was studied in duplicate at initial concentrations of 1.1 and 4.2 μg/ml in 30 ml of the preequilibrated perfusion medium in 250-ml Erlenmeyer flasks kept at 37°C and 50 oscillations/min in a water bath shaker (Ebecbach, Ann Arbor, Mich.). Dx stock solution, 0.3 to 0.4 ml, was pipetted into the flask followed by a brief stirring with a glass rod. About 1 ml of sample was quickly collected at 0.17, 0.33, 0.5, 0.67, 1, 1.5, 2, 3, 5, 7, 10, 15, 20, 30, and 60 min into ice-cooled microcentrifuge tubes and centrifuged immediately for 20 sec at 3000 rpm. Two sets of 200 μl of plasma samples were stored in a freezer until assay. The effect of heparin (5 U/ml) on the influx of Dx into RBC was also studied since it had been reported that Dx could be bound to heparin (18,19).

HPLC Assay

For plasma analysis, 100 μl of 30% trichloroacetic acid (TCA) was added to 200 μl of samples in microcentrifuge tubes. After 5 sec of vortex mixing, they were centrifuged for 2 min at 3000 rpm and 50 μl of the supernatant was injected onto the column within 5 min. For blood analysis, 100 μl of water was added to 200 μl of samples followed by 5 min of sonication in an ultrasonicator before the addition of TCA. The above assay was protected from light to prevent possible degradation (10). When plasma and blood samples were exposed to light, the decrease in Dx concentration was less than 5% in 1 hr.

The HPLC system consisted of a solvent delivery pump (Model 110A, Beckman Instruments, Berkeley, Calif.), a syringe loading sample injector (Model 7125, Rheodyne, Cotati, Calif.), a 3.9 mm (I.D.) × 30-cm 10-μm μBondapak alkylphenyl column (Waters Associates, Milford, Mass.), a fluorescence detector (Model SF-970, Schoeffel Instruments, Westwood, N.J.), and a 10-mV potentiometric 10-in.

recorder (Linear Instruments, Irvine, Calif.). In order to reduce noise and improve sensitivity, a filter/amplifier (Model 1021A, Spectrum, Newark, Del.) was employed. The mobile phase was 30% acetonitrile in water acidified to pH 3.0 with phosphoric acid and run at 2 ml/min at ambient temperature. The excitation wavelength was set at 470 nm and an emission filter with a cutoff near 585 nm was used (20).

Standard curves from spiked Dx concentrations of 0.16, 0.32, 0.79, 2.01, 3.16, and 6.32 μg/ml in plasma and of 0.79, 1.58, 2.01, 3.16, 6.32, and 12.65 μg/ml in blood (perfusion medium) were prepared. In each experiment, daily working standards were prepared for both plasma and blood assays at concentrations of 0.1, 1, and 3 μg/ml (0.1 μg/ml in blood was not used). For reproducibility studies, aliquots (200 μl) of plasma or blood were spiked to yield 0.1, 1, and 3 μg/ml in plasma and 1, 3, and 5 μg/ml in blood and assayed in quadruplicate using the method described.

Data Analysis

The hepatic extraction ratio (E) was calculated by

$$E = (C_{in} - C_{out})/C_{in} \quad (1)$$

where C_{in} is the mean of two or three inlet blood concentrations measured, and C_{out} is the mean of five steady-state outlet blood concentrations measured.

The fraction of drug present in plasma (F_p) of the inlet or outlet blood was calculated according to the following equation:

$$F_p = (1 - HC)/(B/P) \quad (2)$$

where HC is the hematocrit and B/P is the blood-to-plasma Dx concentration ratio.

The fraction of drug present in RBC (F_r) was calculated by

$$F_r = 1 - F_p \quad (3)$$

The ratio (P/R) of plasma drug concentration to RBC drug concentration was calculated by

$$P/R = (HC/F_r)/(B/P) \quad (4)$$

The net fractional removal of drug from plasma of the inlet blood (E_p) was calculated by

$$E_p = (C_{in,p} - C_{out,p})/C_{in,p} \quad (5)$$

where $C_{in,p}$ is the mean of two or three inlet plasma concentrations measured, and $C_{out,p}$ is the mean of five steady-state outlet plasma concentrations measured.

The net fractional removal of drug from RBC of the inlet blood (E_r) was calculated by

$$E_r = (C_{in,r} - C_{out,r})/C_{in,r} \quad (6)$$

where $C_{in,r}$ is the mean of two or three calculated ($C_{in} \times F_r/HC$) inlet RBC concentrations, and $C_{out,r}$ is the mean of five calculated ($C_{out} \times F_r/HC$) steady-state outlet RBC concentrations.

The statistical differences of E, B/P, and P/R ratios of the inlet and outlet blood between condition I and condition II in six rats were evaluated using Student's paired t test according to SAS (21).

RESULTS

HPLC Assay

The simple HPLC assay described above was adequate for the present study. Typical chromatograms from blank plasma and blood samples, samples spiked with known Dx concentrations, and actual samples from perfusion studies are shown in Fig. 1. The standard curve was linear over the concentration range studied, with an r value of 0.9998 for plasma samples and 0.9984 for blood samples. The detection limits for plasma and blood based on a signal-to-noise ratio of 3 were 0.049 and 0.5 μg/ml, respectively. The determination of Dx was highly reproducible, with a CV of less than 3% for all concentrations examined.

A major known metabolite, doxorubicinol, was not detected, probably because of extensive biliary secretion (9). This metabolite, if present, would not interfere with our assay as shown in Fig. 1.

Although various HPLC methods for the assay of Dx in plasma have been published (20,22,23), they usually require larger volumes of samples and more time-consuming extraction steps. No HPLC methods for analysis of blood samples appear to have been published.

Stability of the Perfusion System

The stability of the liver perfusion system was established during preliminary investigations. For example, the O_2 concentration of the inlet (about 14 ppm) or the outlet (4–6 ppm) blood was practically constant in each rat study. The outlet plasma or blood Dx concentration was also fairly constant between 10 and 80 min during an infusion study (Fig. 2). Constancy in O_2 consumption and E was then used to judge liver stability during subsequent studies.

Hepatic Extraction Ratio Under Conditions I and II

Typical C_{out} profiles of Dx are shown in Fig. 3. There was a marked difference in C_{out} between condition I and condition II in spite of their similar C_{in} values. Individual E values from the six rats are summarized in Table I. The mean E values under conditions I and II were 0.286 ± 0.131 (SD)

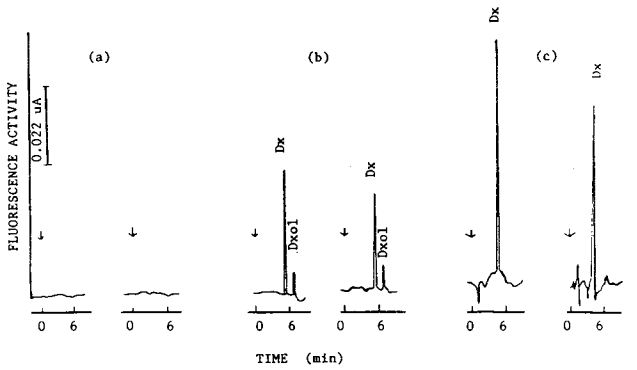

Fig. 1. HPLC chromatograms: (a) blank plasma (left) and blood (right); (b) blank plasma (left) and blood (right) spiked with 0.32 and 0.79 μg/ml of doxorubicin (Dx) doxorubicinol (Dxol); (c) plasma (left) and blood (right) from the outlet blood of an isolated liver preparation after Dx infusion under condition I (arrows indicate injection time).

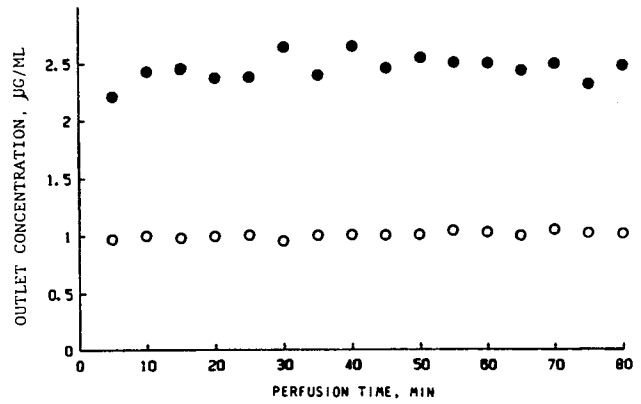

Fig. 2. Outlet plasma (○) and blood (●) doxorubicin concentration profiles during an 80-min constant infusion to a liver under condition I.

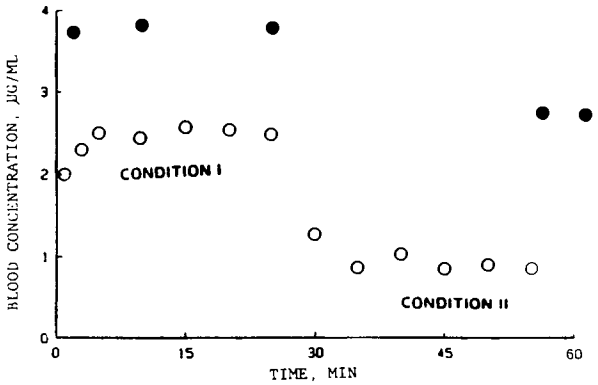

Fig. 3. Typical inlet (●) and outlet (○) blood doxorubicin concentration profiles obtained under conditions I and II (rat 2).

Table I. Hepatic Extraction Ratio of Doxorubicin Studied Under Conditions I (E_I) and II (E_{II})

Rat	E_I	E_{II}	% difference[a]
1	0.102	0.586	474
2	0.365	0.657	80
3	0.186	0.422	127
4	0.313	0.676	116
5	0.473	0.789	67
6	0.279	0.420	51
Mean ± SD	0.286 ± 0.131	0.592 ± 0.147	152 ± 160

[a] % difference = $[(E_{II} - E_I)/E_I] \times 100$.

and 0.592 ± 0.147, respectively. The increase in E from condition I to condition II ranged from 51% (rat 6) to 474% (rat 1) with a mean difference of 152% ($P < 0.001$).

Distribution of Doxorubicin in the Inlet and Outlet Blood

Mean B/P, F_p and P/R in six rats obtained during the infusion are summarized in Table II. Under condition I, differences in B/P ($P < 0.02$), F_p ($P < 0.002$), and P/R ($P < 0.0003$) between the inlet and the outlet blood were all statistically significant. Under condition II, differences in B/P ($P > 0.5$) and P/R ($P > 0.1$) were not significant, while the difference in F_p ($P < 0.02$) was significant. For inlet blood between two conditions, the differences in B/P, F_p, and P/R were all significant ($P < 0.003$).

Analysis of Removal of Doxorubicin from Plasma and RBC

Mean fractional removal of Dx from plasma (E_p) and RBC (E_r) between condition I and condition II in six rats are summarized in Table III. A larger fraction of Dx was removed from plasma than from RBC under condition I. The mean E_p values between the two conditions were not significantly different ($P > 0.66$). Higher E_r values obtained in condition II are probably an artifact because of relatively small amounts of Dx present in the RBC and the lag time involved in sample collection and centrifugation.

Storage Effect of Doxorubicin

Typical rising (condition I) and descending (condition II) plasma concentration profiles of Dx in outlet blood obtained during a steady-state infusion and centrifuged after different lengths of times of standing are shown in Fig. 4. Individual differences in plasma concentration between zero time and 15 min in six rats are summarized in Table IV. The mean differences for conditions I and II were 81 ± 69 and 137 ± 100%, respectively. The B/P of Dx in the outlet blood decreased ($P < 0.05$) from 3.53 ± 1.08 to 2.01 ± 0.52 after 15 min of standing for condition I and increased ($P < 0.05$) from 0.89 ± 0.10 to 2.03 ± 1.06 for condition II. The mean B/P values of the outlet blood obtained after 15 min of standing are also given in Table II.

Influx Rate of Doxorubicin

The plasma Dx concentration versus blood incubation time profiles in the *in vitro* influx study are shown in Fig. 5. The equilibration appears to occur quite rapidly, approximately 1 to 1.5 and 2 to 2.5 min at initial blood concentra-

Table II. Distribution of Doxorubicin in the Inlet and Outlet Blood During Steady-State Infusion Under Conditions I and II in Six Rats

		Condition I							Condition II						
		Inlet blood			Outlet blood				Inlet blood			Outlet blood			
	HC	B/P	F_p	P/R	B/P	F_p	P/R	B/P^a	B/P	F_p	P/R	B/P	F_p	P/R	B/P^a
Mean	0.27	2.25	0.35	0.19	3.53	0.22	0.11	2.01	0.92	0.79	1.96	0.89	0.85	2.47	2.03
SD	0.03	0.27	0.05	0.04	1.08	0.05	0.03	0.52	0.20	0.09	1.35	0.10	0.07	1.40	1.06

[a] B/P obtained after 15 min of standing of the outlet blood at room temperature; all other data are based on results obtained from immediately centrifuged and separated plasma.

Table III. Mean Values of Fractional Removal of Doxorubicin from Plasma (E_p) and Erythrocytes (E_r) Under Conditions I and II in Six Rats

	Condition I		Condition II	
	E_p	E_r	E_p	E_r
Mean	0.537	0.171	0.571	0.680
SD	0.140	0.117	0.151	0.147

tions of 1.1 and 4.2 μg/ml, respectively. A plotting of fractional change in plasma Dx concentration [(plasma concentration at a given time − plasma concentration at equilibrium)/(theoretical plasma concentration at time zero − plasma concentration at equilibrium)] versus incubation time (24) was made on a semilog paper (not presented here). The times for the initial 50% change in plasma concentration were estimated to be about 0.5 and 0.7 min for the low and high concentration studies, respectively. The influx profile was not altered in the presence of heparin.

DISCUSSION

The present data (Table I) show that differences in preparing the Dx perfusion medium prior to infusion markedly affect hepatic elimination; the maximum difference in E is about sixfold (rat 1). These findings contradict the assumptions of most conventional hepatic models (1) that equilibration of drug between plasma and RBC is instantaneous. Therefore, the inlet blood studied under conditions I and II would be considered to be identical and should result in an identical hepatic extraction. The present findings, however, support the hypothesis (1) that the degree of equilibration of drug between plasma and RBC in blood prior to entering the liver greatly affects E because of the relatively slow diffusion of drug across the RBC membrane as compared to the blood transit time in the liver. In the present study a greater drug fraction should be present in the plasma of inlet blood under condition II than under condition I. Therefore, the E from condition II will be higher than from condition I. The above contention is supported by data shown below.

Table IV. Plasma Doxorubicin Concentration of the Steady-State Outlet Blood Centrifuged After Zero Time and 15 min of Standing at Room Temperature

Rat No.	Condition I			Condition II		
	$C_{out,0}$	$C_{out,15}$	% diff[a]	$C_{out,0}$	$C_{out,15}$	% diff[b]
1	0.83	—[c]	—[c]	1.30	0.86	52
2	0.84	1.48	75	1.73	0.87	98
3	0.57	0.89	55	1.33	0.85	56
4	0.39	0.58	51	0.49	0.26	93
5	0.27	0.81	201	0.31	0.09	246
6	0.89	1.10	24	1.47	0.39	280
Mean	0.63	0.97	81	1.10	0.55	137
S.D.	0.26	0.34	69	0.57	0.35	100

[a] % diff = $[(C_{out,15} - C_{out,0})/C_{out,0}] \times 100$.
[b] % diff = $[(C_{out,0} - C_{out,15})/C_{out,15}] \times 100$.
[c] Sample was not collected.

The difference in the degree of equilibration of Dx between plasma and RBC of the inlet blood is clearly illustrated (Table II) by the greater F_p (0.79 vs 0.35) or lower (0.92 vs 2.25) B/P ratio obtained under condition II compared to under condition I. The actual difference may be much larger because of the lag time in sample collection and centrifugation, especially for condition II; some drug in plasma might be still taken up by RBC during this time, resulting in a lower F_p or a higher B/P. The initial difference in the distribution of Dx in inlet blood is apparently the major factor for causing markedly different hepatic extraction between condition I and condition II.

The slow efflux of Dx from RBC into plasma during the perfusion study is evident from the significant reduction in the P/R ratio (0.19 vs 0.11) of the outlet blood compared to the inlet blood studied under condition I (Table II); again, the actual difference might be higher because of the lag time in sample collection and centrifugation (i.e., some drug from RBC might diffuse into plasma during this period, resulting in a higher P/R ratio). This is also supported by the rising plasma Dx concentration of the outlet blood under condition I when it was stored for various times at room temperature

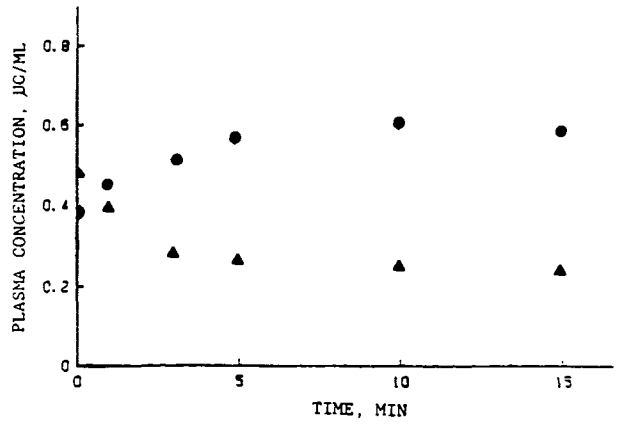

Fig. 4. Typical plasma doxorubicin concentration profiles of the steady-state outlet blood obtained under conditions I (●) and II (▲) and centrifuged after different times of standing at room temperature (rat 5).

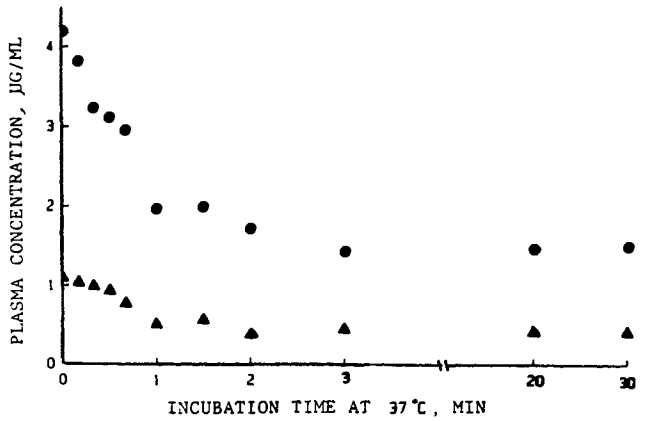

Fig. 5. The plotting of plasma doxorubicin concentration vs incubation time at initial blood concentrations of 1.1 (▲) and 4.2 (●) μg/ml from the influx rate study.

(Fig. 4). The mean difference (Table IV) in plasma concentration between immediately centrifuged and apparently equilibrated blood samples (stored at room temperature for 15 min) is about 81%, and the real difference may also be larger. The rising plasma concentration pattern observed (Fig. 4) is unlikely attributed to a change in P/R ratio between 37°C and room temperature because of a lack of difference in B/P between inlet blood kept at 37°C and outlet blood kept at room temperature under condition I (2.25 ± 0.87 vs 2.01 ± 0.52; $P > 0.1$) or II (2.25 ± 0.87 vs 2.03 ± 1.06; $P > 0.5$) (Table II). The postulated slow efflux in the present study is also consistent with the reported (25) *in vitro* Dx study showing that at 1–6 μM concentrations, similar to that employed in our study, the apparent efflux half-time from human RBC is about 1.8 min. The above discussions indicate that most of the Dx in the RBC of inlet blood under condition I was not available for hepatic elimination. This is also consistent with markedly different E_p (a mean of 0.537) and E_r (a mean of 0.171; the actual value may be much lower) values obtained under condition I (Table III).

The relatively slow influx of Dx from plasma into RBC was demonstrated by the difference (0.19 vs 1.96) in the P/R ratio of the inlet blood between condition I and condition II (Table II). This is also supported by the declining plasma concentration pattern when the outlet blood under condition II was stored at room temperature (Fig. 4 and Table IV). The relative slowness of influx is also evident from the present *in vitro* study (Fig. 5). At the two Dx concentrations studied, the initial influx half-times were approximately 0.5 and 0.7 min, respectively, which are several times longer than the mean hepatic transit time of blood. If intensive sampling during the first 2 min was not carried out, one might conclude that the distribution equilibration of Dx between plasma and RBC is extremely rapid or instantaneous, and thus, the distribution factor may not be important in hepatic modeling. Intensive initial blood sampling, especially during the first 1 or 2 min, has not been carried out in most studies on plasma/RBC distribution of drugs reported to date (26–28).

Most drug molecules delivered to the liver after intravenous administration should be more equilibrated in the blood than after oral administration during the first pass (1), because the mean transit time of drug between the absorption site and the liver (probably less than 1–2 sec) (1) is shorter than the mean transit time of drug in blood circulation after intravenous administration ($\bar{t}$). The $\bar{t}$ may be estimated by dividing the total blood volume in the body by the blood clearance (29). Therefore, the present experimental design under condition I may more closely simulate the intravenous study while that under condition II may more resemble oral administration. Hence, the degree of hepatic first-pass metabolism of drugs showing a slow distribution between plasma and RBC may be considerably underestimated from intravenous blood data using the standard method (30–32). The above hypothesis is consistent with occasional reports of lower oral bioavailability than predicted. For example, the oral bioavailability of tiodazocine in dogs predicted from intravenous study (33) was approximately three times higher than the actual bioavailability (0.71 vs 0.26). Obviously, the above lower than predicted bioavailability may also be caused by incomplete absorption and/or gut wall metabolism (33).

In our study, the E_p under condition I is almost the same as the E under condition II ($P > 0.66$). This result could suggest that plasma data rather than blood data from the intravenous or oral administration might be more useful in predicting the hepatic first-pass effect for drugs showing slow distribution properties in blood. The oral bioavailability of tiodazocine in dogs (33) predicted from plasma data (1 − hepatic plasma clearance/hepatic plasma flow) was indeed closer to the observed bioavailability (0.17 vs 0.26). Further studies in this area appear worthwhile.

The present Dx data are consistent with earlier studies on renally excreted drugs such as diodrast (4) and *p*-aminohippuric acid (5) in dogs. These drugs in RBC were not as readily available for renal elimination as in plasma during intravenous infusion. This was demonstrated by their higher P/R ratios in arterial renal blood than in venous renal blood and by their increasing P/R ratios of venous blood upon standing at 37°C (4,5).

The above discussions, together with recently reported unusual distribution profiles in blood of many drugs such as methotrexate (34), furosemide (35), and procainamide (36), present additional complexities (3,37) in our understanding of hepatic handling of drugs. In the present study, human plasma was not used because of its cost and its unnecessity for illustrating the effect of RBC as barriers for hepatic elimination. The protein binding in plasma (38) should generally reduce the E or E_p (1,3,17,37) and would probably reduce the degree of difference in E obtained between condition I and condition II. Further studies are obviously needed.

REFERENCES

1. W. L. Chiou. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **22**:577–590 (1984).
2. C. A. Goresky, G. G. Bach, and B. E. Nadeau. *Circ. Res.* **36**:328–351 (1975).
3. G. R. Wilkinson. *Pharmacol. Rev.* **39**:1–47 (1987).
4. H. L. White. *Am. J. Physiol.* **130**:454–463 (1940).
5. R. A. Phillips, V. P. Dole, P. B. Hamilton, K. Emerson, R. M. Archibald, and D. D. Van Slyke. *Am. J. Physiol.* **145**:314–336 (1946).
6. L. S. Schanker, P. A. Nafpliotis, and J. M. Johnson. *J. Pharmacol. Exp. Ther.* **135**:325–331 (1961).
7. M. Dalmark. *Scand. J. Clin. Lab. Invest.* **41**:633–639 (1981).
8. W. L. Chiou. *J. Pharm. Sci.* **72**:1365–1368 (1983).
9. N. Tavoloni and A. M. Guarino. *Pharmacology* **21**:244–255 (1980).
10. S. Eksborg, H. Ehrsson, and I. Anderson. *J. Chromatogr.* **164**:479–486 (1979).
11. S. Pang. In J. R. Mitchell and M. G. Horning (eds.), *Drug Metabolism and Drug Toxicity*, Raven Press, New York, 1977, pp. 331–352.
12. L. L. Miller. In I. Bartosek, A. Guaitani, and L. L. Miller (eds.), *Isolated Liver Perfusion and Its Applications*, Raven Press, New York, 1973, pp. 1–66.
13. F. G. Collins and J. L. Skibba. *J. Surg. Res.* **28**:65–70 (1980).
14. H. Boxenbaum. *J. Pharmacokin. Biopharm.* **8**:165–176 (1980).
15. A. R. Beaubien, L. Tryphonas, A. P. Pakuts, M. Macconaill, and H. A. Combley. *J. Pharmacol. Methods* **2**:213–221 (1979).
16. J. L. Skibba, E. J. Frank, and R. E. Condon. *Cancer Treat. Rep.* **66**:1357–1363 (1982).
17. K. S. Pang and M. Rowland. *J. Pharmacokinet. Biopharm.* **5**:655–680 (1977).

18. E. J. Modest, G. T. Brandt, W. R. Shehee, and F. Prosl. *Proc. Annu. Meet. Am. Soc. Clin. Oncol.* 3:34 (1984).
19. T. Colombo, F. Delaini, R. Ferrari, M. B. Donati, M. G. Donelli, and A. Poggi. *Biomedicine* 34:124–128 (1981).
20. N. R. Bachur, A. L. Moore, J. G. Bernstein, and U. Liu. *Cancer Chemother. Rep. Part 1* 54:89–94 (1970).
21. SAS: *Sas User's Guide. Statistics, Version 5 Edition.* SAS Institute, Cary, N.C., 1985.
22. R. S. Benjamin, C. E. Riggs, Jr., and N. R. Bachur. *Cancer Res.* 37:1416–1420 (1977).
23. M. J. M. Oosterbaan, R. J. M. Dirks, T. B. Vree, and E. V. D. Kleijn. *J. Chromatogr.* 306:323–332 (1984).
24. A. Yayon and H. Ginsburg. *Biochim. Biophys. Acta* 686:197–203 (1982).
25. M. Dalmark and H. H. Storm. *J. Gen. Physiol.* 78:349–359 (1981).
26. S. M. Wallace and S. Riegelman. *J. Pharm. Sci.* 66:729–731 (1977).
27. N. K. Athanikar and W. L. Chiou. *J. Pharmacokin. Biopharm.* 7:383–397 (1975).
28. M. G. Lee, C. Y. Lui, M. L. Chen, and W. L. Chiou. *Int. J. Clin. Pharmacol. Ther. Toxicol.* 22:530–537 (1984).
29. W. L. Chiou. *Clin. Pharmacokinet.* 17:175–199 (1989).
30. M. Gibaldi and D. Perrier. *Pharmacokinetics,* Marcel Decker, New York, 1982.
31. S. M. Huang, N. K. Athanikar, K. Stidhar, Y. C. Huang, and W. L. Chiou. *Eur. J. Clin. Pharmacol.* 22:359–365 (1982).
32. W. L. Chiou. *J. Pharmacokinet. Biopharm.* 3:193–201 (1975).
33. R. A. Baughman, B. A. Mico, and L. Z. Benet. *Drug Metab. Dist.* 11:143–146 (1982).
34. M. G. Lee, C. Y. Lui, and W. L. Chiou. *Biopharm. Drug Dispos.* 7:487–494 (1986).
35. M. G. Lee, M. Chen, and W. L. Chiou. *Res. Commun. Chem. Pathol. Pharmacol.* 34:17–28 (1981).
36. M. Chen, M. G. Lee, and W. L. Chiou. *J. Pharm. Sci.* 72:572–574 (1983).
37. W. L. Chiou. *Res. Commun. Chem. Pathol. Pharmacol.* 6:121–124 (1987).
38. P. A. Harris and J. F. Gross. *Cancer Chemother. Rep. Pt. 1* 59:819–825 (1975).