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Report

Erythrocytes as Barriers for Drug Elimination in the Isolated Rat Liver. II. Propranolol

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The potential of erythrocytes (RBC) to serve as "barriers" of hepatic elimination of propranolol, a drug with rapid equilibration in blood, was studied in rats under two conditions: (I) the drug was preequilibrated in blood before infusion into the liver, and (II) the drug was directly infused into the liver. The mean fractions of dose escaping elimination during each pass under conditions I and II were 0.0561 ± 0.040 and 0.0290 ± 0.024, respectively ($P < 0.02$). Contrary to the early study on doxorubicin, most drug molecules in RBC were found to be available for elimination. Implications of the present findings in the prediction of hepatic first-pass effect after oral administration, on the basis of intravenous data, are discussed. Marked underestimation of oral bioavailability of propranolol in humans is consistent with the RBC "barrier" effect hypothesis.

KEY WORDS: erythrocytes barrier; hepatic first-pass effect; isolated rat liver; propranolol.

INTRODUCTION

In the preceding paper (1), erythrocytes (RBC) were shown to have a marked "barrier" effect on doxorubicin elimination in isolated perfused rat liver preparations. The mean difference in hepatic extraction ratio (E) between condition I, in which the drug was preequilibrated in "blood" before infusion into the liver, and condition II in which the drug was directly infused into the liver, was 152 ± 160% (SD). The above result has been attributed to the fact that the equilibration of drug between plasma and RBC is relatively slow compared to the blood transit time (about 10 sec) in the liver (2–4). Therefore, the drug in RBC, in either free or bound form, is not as available for hepatic elimination as the drug in plasma. The slow distribution of doxorubicin in blood was demonstrated (1) by marked differences in the plasma/RBC ratio of the inlet blood between condition I and condition II (0.19 vs 1.96), by the slow *in vitro* influx of the drug from plasma into RBC, and by the slow efflux of the drug from RBC into plasma.

Propranolol was chosen in the present study because it equilibrates more rapidly in blood as judged from a preliminary *in vitro* investigation (to be detailed later). Therefore, it is of interest whether the "RBC barrier" effect also occurs with such a compound.

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METHODS

Influx Rate of Propranolol from Plasma into Erythrocytes

This was similarly (1) studied in duplicate at initial propranolol (kindly supplied by Ayerst Lab, New York) blood concentrations of 0.025 and 2.5 μg/ml.

Perfusion of Propranolol in Isolated Rat Livers

Propranolol was infused at about 29 μg/min to six livers in a crossover fashion under two conditions (1). Two sets of outlet "blood" samples were collected every 4 to 5 min during infusion. In rats 2 and 3 one set of blood samples was immediately centrifuged to separate "plasma" to see any difference in distribution kinetics of propranolol between the inlet and the outlet blood under conditions I and II and between the inlet blood under condition I and that in condition II . In rats 5 and 6, plasma data were used to estimate the E value because in initial studies the blood-to-plasma drug concentration ratio (B/P) of the inlet blood was practically the same as that in the outlet blood. Propranolol was quantitated by a high-performance liquid chromatographic (HPLC) method developed earlier for human plasma (6).

Data Analysis

The (F) of propranolol escaping elimination during each single passage through the liver and the E were calculated from the input (C_{in}) and the output (C_{out}) concentration data described earlier (1). The equation to calculate the fractional removal of drug from the RBC of inlet blood during a single passage (E_r) under condition I was derived from Eq. *(1)* based on the mass-balance theory (3) as shown below:

$$E = E_p F_p + E_r F_r \qquad (1)$$

where E_p is the fractional removal of drug from plasma of the inlet blood, F_p is the fraction of drug present in the plasma of the inlet blood and calculated based on Eq. *(2)* in the previous study (1), and F_r is the fraction of drug present in the RBC of blood. The E value under condition II was used as an approximation for E_p. This appears reasonable in view of the extremely short contact period of the drug with the RBC prior to entering the liver under condition II (probably less than 1 sec) and the very efficient hepatic extraction for this drug (discussed later); thus all drug molecules may be confined to the plasma of the inlet blood. Since $F_r = 1 - F_p$, Eq. (1) can be rewritten as

$$E_r = (E - E_pF_p)/(1 - F_p) \quad (2)$$

The fraction of drug unbound in RBC of the inlet blood under condition I ($f_{u,r}$) was estimated by (7)

$$f_{u,r} = C_{in,p} \times 0.7/C_{in,r} \quad (3)$$

where $C_{in,p}$ and $C_{in,r}$ are inlet plasma and RBC concentrations, respectively. The above equation assumes that at equilibrium the plasma drug concentration will be the same as the unbound drug concentration in RBC because of the lack of plasma proteins employed in the present study. A correction factor of 0.7 was used since the actual cellular water volume is about 70% of the RBC (8). If plasma sample was not collected, $C_{in,p}$ was calculated by

$$C_{in,p} = C_{in}/(B/P) \quad (4)$$

where the B/P value is the average blood/plasma concentration ratio obtained from the preliminary study. The $C_{in,r}$ was estimated by

$$C_{in,r} = [C_{in} - C_{in,p}(1 - HC)]/HC \quad (5)$$

where HC is the hematocrit.

RESULTS AND DISCUSSION

Distribution Kinetics of Propranolol in Blood

The plasma propranolol concentration versus blood incubation–time profiles are shown in Fig. 1. The time to reach equilibrium in blood was apparently too rapid to be determined (<20 sec). This is in sharp contrast with the doxorubicin study (1), in which apparent distribution equilibrium was reached at about 1 to 1.5 and 2 to 2.5 min at initial concentrations of 1.1 and 4.2 μg/ml, respectively. The B/P ratios from the present two studies, with a 100-fold difference in drug concentration, were essentially identical (1.92 vs 2.04). The distribution kinetics were not altered in the presence of 5 units/ml of heparin. The B/P ratios from the inlet blood under conditions I and II, the outlet blood under conditions I and II, and the preliminary stability study were all similar, with a grand mean of 2.17 ± 0.17, in spite of high hepatic extraction (E being about 0.944 under condition I and 0.971 under condition II as shown in Table I). The rapid and concentration-independent distribution of propranolol in blood is also in sharp contrast with the previous study (1).

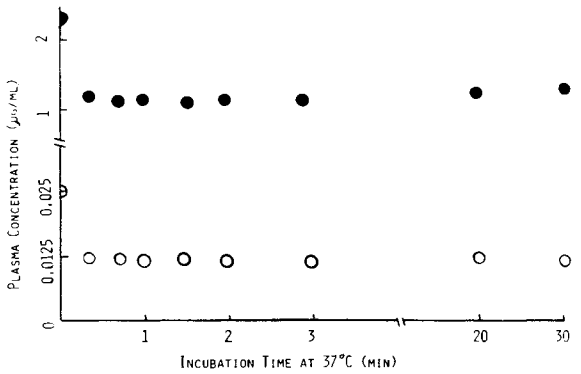

Fig. 1. The plotting of plasma propranolol concentration vs incubation time at initial blood concentrations of 0.025 (○) and 2.5 (●) μg/ml from the influx rate study.

Hepatic Extraction and Bioavailability Under Conditions I and II

The stability of the liver preparation employed in the present study was demonstrated by fairly constant C_{out} values between 5 and 90 min in a preliminary constant-infusion study (Fig. 2) and subsequent studies under conditions I and II (Fig. 3). There was a marked difference in the C_{out} between condition I and condition II in spite of their similar C_{in}. Individual E and F values are listed in Table I. The mean E values under conditions I and II were 0.944 ± 0.040 (SD) and 0.971 ± 0.024, respectively. The difference in E between the two conditions studied ranged from 0.71% (rat 5) to 5.26% (rat 3), with a mean difference of 2.88% ($P < 0.02$). The corresponding mean F values were 0.0561 ± 0.0401 and 0.0290 ± 0.024, respectively. The difference in F between the two conditions ranged from 35% (rat 4) to 272% (rat 2), with a mean difference of 107% ($P < 0.02$).

Rationale for Difference in Elimination Under Conditions I and II

The difference in elimination of propranolol observed under conditions I and II may be twofold. First, the time to reach the equilibrium of propranolol in the inlet blood under condition II might be longer than that required for drug molecules to travel from infusion site to the liver (probably less than 1 sec). This could result in a difference in the initial distribution of propranolol between plasma and RBC in the inlet blood between condition I and condition II. Therefore, under condition II more drug molecules would be present in

Table I. Hepatic Extraction Ratio (E) and Bioavailability (F) of Propranolol Studied Under Condition I and Condition II

Rat	E_I	E_{II}	% difference in E^a	F_I	F_{II}	% difference in F^b
1	0.968	0.982	1.45	0.0323	0.0185	75
2	0.944	0.985	4.34	0.0562	0.0151	272
3	0.894	0.941	5.26	0.1060	0.0595	78
4	0.974	0.981	0.72	0.0258	0.0191	35
5	0.987	0.994	0.71	0.0130	0.0064	108
6	0.897	0.940	4.79	0.1030	0.0603	71
Mean	0.944	0.971	2.88	0.0561	0.0298	107
S.D.	0.040	0.024	2.14	0.040	0.024	84

[a] % difference in $E = [(E_{II} - E_I)/E_I] \times 100$.
[b] % difference in $F = [(F_I - F_{II})/F_{II}] \times 100$.

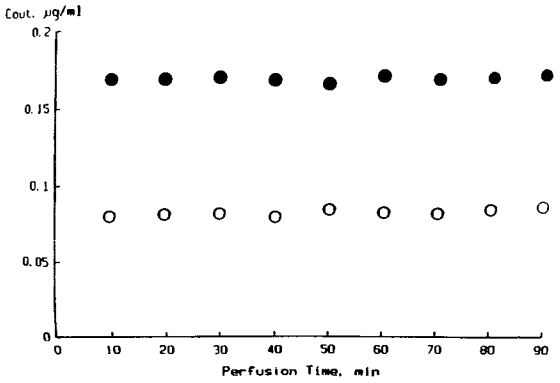

Fig. 2. Outlet plasma (○) and blood (●) propranolol concentration profiles during a 90-min constant infusion to a liver under condition I.

plasma and available for elimination. On the other hand, the drug in the RBC of the inlet blood under condition I might not be as available for hepatic elimination as in the plasma due to relatively slow efflux of drug from RBC to plasma as compared to hepatic blood transit time. This condition may be reflected by the calculated lower ($P < 0.022$) E_r value (0.931 ± 0.049; $N = 6$) than either the mean E (0.944 ± 0.040) or the mean E_p (0.971 ± 0.024) values (i.e., E_{II} in Table I). Consequently, lower E would be obtained under condition I than under condition II. Since most drug molecules in RBC are in bound form ($84.9 \pm 1.0\%$; $N = 6$), the present results of high E_r values would also indicate that both the bound and the unbound fractions of the drug in RBC are available (although not completely) for elimination.

While the distribution of propranolol in blood was fast as reflected by the similar B/P ratios in the inlet blood between condition I and condition II (2.18 ± 0.10 and 2.32 ± 0.06), our failure to detect any difference may be due to unavoidable lag time in sample collection and centrifugation. Thus, the present study indicates that the "barrier effect" could also occur with a drug which appears to equilibrate rapidly in blood.

Implications in Hepatic First-Pass Metabolism

The results of the present study might indicate that the prediction of oral bioavailability of propranolol from intravenous blood data is not very accurate if one considers that conditions I and II employed may be simulating intravenous and oral administration, respectively (1). Therefore, the use of intravenous blood data may underestimate the hepatic first-pass effect and overestimate the oral bioavailability (1,3). This contention seems to be supported by the results of our analysis of some reported propranolol data. For example, after eight volunteers received 80 mg orally and 2.2 mg intravenously in a crossover fashion (9), the predicted mean absolute bioavailability (F_{pred}) using the following standard technique (10–12) is three times higher than the observed bioavailability (0.51 vs 0.18).

$$F_{pred} = 1 - \frac{f_m \times Cl_{iv}}{Q} \quad (6)$$

where f_m is the fraction of the intravenous dose metabolized by the liver, Q is the liver blood flow, and Cl_{iv} is the blood clearance [i.e., plasma clearance × (P/B)]. In the above calculation a Q of 1.5 liters/min (13), a mean B/P value of 0.77 (14), and an f_m of 1 (15,16) were used. In another study following simultaneous intravenous (as [^{3}H]propranolol) and oral (as unlabeled 80 mg of the drug) administration to six human subjects (17), the mean F_{pred} can be estimated to be 1.5 times higher than the observed bioavailability (0.34 vs 0.22). If one considers the nonlinear hepatic elimination of propranolol (18,19), the actual bioavailability at doses in the linear range may be even lower than those (0.18 and 0.22) reported (9,17). The absorption of propranolol from the GI tract is virtually complete (15).

If one uses the reported (9) intravenous *plasma* clearance data for estimation of E after oral dose (i.e., E is equal to hepatic plasma clearance divided by hepatic plasma flow; hematocrit is assumed to be 0.45), the predicted bioavailability (0.29) is closer to the observed bioavailability (0.18). In such an analysis all the drug in RBC is assumed not to be available for elimination; this assumption is not entirely consistent with the present findings, which showed that most propranolol molecules in RBC are also available for elimination. The present study, using outdated human erythrocytes in rats, did not exactly mimic the *in vivo* condition in humans. Nevertheless, the results of using plasma data to predict the hepatic first-pass effect more accurately for propranolol in humans and for tiodazocine in dogs (1) suggest a need for further studies with other drugs.

Unpredictable variability in steady-state plasma propranolol concentrations and oral bioavailability has often been reported (20–22). Variabilities in liver blood flow, intrinsic hepatic clearance, and plasma protein binding have been mentioned as possible causes (20,21). In view of the present study, individual differences in the degree of equilibration of propranolol in blood before reaching the liver might be an additional contributing factor.

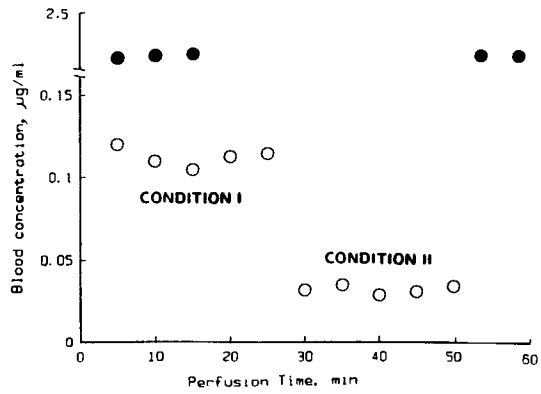

Fig. 3. Typical inlet (●) and outlet (○) blood propranolol concentration profiles obtained under conditions I and II (rat 2).

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