Research Article

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

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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-
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% O₂–5% CO₂ 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 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 O₂ consumption of the inlet and outlet blood was determined periodically with an O₂ 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 Bondapak alkylphenyl column (Waters Associates, Milford, Mass.), a fluorescence detector (Model SF-970, Schoeffel Instruments, Westwood, N.J.), and a 10-nV potentiometric 10-in.