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RESEARCH ARTICLE

Hepatic uptake of epirubicin by isolated rat hepatocytes and its biliary excretion after intravenous infusion in rats

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Abstract Anthracycline anticancer agents are widely used in the cancer chemotherapy for hepatocelluar carcinoma. However, accurate kinetic analyses of the hepatocellular uptake and efflux of the drugs have not been reported. We, therefore, investigated the hepatobiliary transport of epirubicin, an anthracycline derived antibiotic, after intravenous (i.v.) infusion in rats. The hepatic uptake mechanisms of epirubicin were also investigated in isolated rat hepatocytes. To analyze epirubicin levels in the biological samples, we used an HPLC-based method which has been validated for a kinetic study by suitable criteria. The uptake process of epirubicin by the hepatocytes revealed one saturable component, with a K_m of 99.1 μg/mL and V_{max} of 3.70 μg/min/10^6 cells. The initial uptake velocity of epirubicin was significantly inhibited in a temperature-dependent manner. The velocity was also reduced in the presence of metabolic inhibitors such as rotenone or carbonylcyanide-*p*-(trifluoromethoxy)-phenylhydrazone. Substrates for organic anion transporters such as bromosulfophthalein and taurocholate significantly inhibited the initial uptake velocity of epirubicin. We also attempted to determine the hepatobiliary transport of epirubicin after i.v. infusion in vivo. At steady-state after i.v. infusion of epirubicin (10–160 μg/min/kg), the drug was extensively accumulated in the liver, followed by excretion into bile. Furthermore, the $CL_{bile,plasma}$ and $CL_{bile,liver}$ decreased with a corresponding increase in the $C_{ss,plasma}$ and $C_{ss,liver}$. In conclusion, present studies using isolated rat hepatocytes and in vivo i.v. infusion demonstrate that epirubicin is likely to be taken up into liver cells via organic anion transporting polypeptides, and that its biliary excretion might be mediated via specific transporters.

Keywords Epirubicin · Hepatobiliary transport · Isolated hepatocytes · Infusion · Bile excretion

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Introduction

The anthracyclines are some of the most effective anticancer treatments ever developed and are effective against more types of cancer than any other class of chemotherapy agents (Minotti et al. 2004; Peng et al. 1993). Epirubicin, an antineoplastic drug, is favoured over doxorubicin, the most popular anthracycline, in some chemotherapy regimens as it appears to cause fewer side-effects (Berchem et al. 1996; Robert et al. 1993). In the case of cancer chemotherapy for hepatocellular carcinoma, epirubicin is widely used. Thus, determining its hepatobiliary transport is highly useful. Recently, we investigated the pharmacokinetics of epirubicin after an intravenous (i.v.) bolus administration at a dose of 2–50 mg/kg (Shin et al. 2013). The clearance of epirubicin showed nonlinear kinetics at high doses. Such a nonlinearity in the clearance of epirubicin might be attributable to the saturation in the elimination process in hepatobiliary transport (Shin et al. 2013). In our previous study, epirubicin mainly distributed to the liver, kidney and lungs after i.v. bolus administration, as was observed with the other anthracycline antibiotics (Fujita et al. 1986; Iguchi et al. 1980; Yoo et al. 2005; Nagata et al. 2008). Moreover, epirubicin was excreted largely in the bile after i.v. bolus administration (Shin et al. 2013). However, the mechanisms by which epirubicin is selectively excreted in the bile have not been clarified yet.

Hepatic uptake and biliary excretion were considered to be major pathways in the removal of epirubicin (Shin et al. 2013). In addition, it has been reported that carrier-mediated transport contributes to hepatic uptake and biliary excretion (Yamazaki et al. 1996). Thus, saturation of membrane transport proteins in both hepatic uptake and biliary excretion might be one of the factors that cause nonlinear clearance of epirubicin in vivo. We, therefore, have investigated the hepatobiliary transport of epirubicin in rats. The mechanisms of epirubicin uptake were determined in isolated rat hepatocytes. We also investigated the kinetics of hepatobiliary transport after i.v. infusion in vivo. To analyze epirubicin levels in biological samples, we used a simple HPLC-based method which was developed and validated in our laboratory.

Materials and methods

Materials

Epirubicin was obtained from Boryung Pharmaceutical Co. Ltd. (Seoul, Korea). Solvents used in the epirubicin analysis were of HPLC grade and were filtered and degassed just prior to use. All other chemicals used in this study were of analytical reagent grade.

Adult male Sprague–Dawley rats weighing 230–250 g (Sam Tac Co. Ltd., Suwon, Gyeonggi-do, Korea) were used for the kinetic studies. They were housed in individual metabolic cages and maintained under a 12 h light/dark cycle with free access to water.

HPLC analysis of epirubicin levels in biological samples

Epirubicin levels were assayed by reverse phase HPLC on a Luna C_{18} column (4.6 mm × 250 mm, 5 μm) (Phenomenex, Torrance, CA, USA) that was interfaced with a HPLC system (Jasco Co. Ltd., Tokyo, Japan) (Shin et al. 2013). This system consisted of a model PU-980 pump, a model AS-950-10 autoinjector, a model FP-2020 fluorescence detector, and a LC-Net II control Borwin integrator (Jasco Co. Ltd., Tokyo, Japan). The mobile phase was a mixture of 0.02 M NaH_2PO_4 buffer and MeOH (38:62, v/v %). The flow rate was 0.7 mL/min. The epirubicin in elutes was monitored fluorometrically at an excitation wavelength (λ_{ex}) of 480 nm and an emission wavelength (λ_{em}) of 550 nm.

The retention time of epirubicin and the internal standard (I.S.; daunorubicin) were 18.7 and 12.8 min, respectively. The chromatogram shows no peaks that interfere with the epirubicin and I.S. signals. To determine the linearity of the HPLC method, quality control samples were prepared, five for each of nine epirubicin concentrations ranging from 0.01 to 100 μg/mL. These samples were assayed on the day of preparation and on the following four consecutive days. The mean regression equation for plasma was $y = 0.215x - 0.008$ ($r^2 = 0.999$), where y is the peak area ratio and x is the concentration. This equation shows significant linearity ($P < 0.01$) over the concentration range of 0.01–100 μg/mL. The mean regression equations for bile and tissue homogenates were not significantly different from the equation for plasma. Variations for both precision and accuracy of the inter- and intra-day results never exceeded 15 %. The lower limit of quantification (LOQ) was, therefore, defined as 0.01 μg/mL. The mean absolute recovery of epirubicin was over 96 % (Shin et al. 2013).

I.V. infusion of epirubicin and analysis of its plasma and bile levels

Under light pentobarbital sodium anesthesia, the femoral vein and artery were cannulated with PE-50 polyethylene tubing (Intramedic, Clay Adams, Parsippany, NJ, USA.) for epirubicin administration and blood sampling, respectively. A catheter (PE-10, Intramedic, Clay Adams, Parsippany, NJ, USA) was implanted into the bile duct via a small abdominal incision for bile sampling. Epirubicin was infused through the rat femoral vein at a flow rate of either 10, 20, 40, 80 or 160 μg/min/kg. At designated times, blood and bile samples were collected up to 24 h post infusion.

The blood samples were centrifuged for 15 min at 1,500×*g* and the plasma was harvested. Immediately after collecting the plasma (100 μL) samples, daunorubicin (10 μL, 50 μg/mL) was added to each plasma test tube as an internal standard. Methanol (3 mL) was then added to precipitate the proteins and extract the compounds of interest. These mixtures were vortexed for 15 min and centrifuged for 15 min at 1,500×*g*. The supernatants were withdrawn, dried under a stream of dry nitrogen and reconstituted in 150 μL mobile phase for quantitative HPLC analyses. The epirubicin levels in the bile were determined as described above (Shin et al. 2013).

Determination of the liver concentration of epirubicin

The rats were decapitated when the steady-state concentration was reached after continuous i.v. infusion of epirubicin at a flow rate of 10, 20, 40, 80 or 160 μg/min/kg. The liver was immediately removed, blotted onto filter papers, and weighed. The tissues were rinsed in ice-cold 50 mM tris–HCl buffer (containing 0.25 M sucrose, pH 7.4) and homogenized with a glass Potter–Elvehjem-type homogenizer with a Teflon pestle. After extracting 100 μL

of 20 % homogenate with 3 mL of methanol, the concentration of epirubicin in the supernatant was measured as described above (Shin et al. 2013).

Preparation of isolated rat hepatocytes

Hepatocytes were isolated from male rats by the procedure of Iga et al. (1979). After isolation, hepatocytes were suspended (1.7×10^6 cell/ml) at 0 °C in the albumin-free Krebs-Henseleit buffer supplemented with 12.5 mM HEPES (pH 7.4). The viability of isolated cells was determined by 0.4 % trypan blue exclusion test. The value obtained usually ranged from 95 to 98 %.

Uptake of epirubicin by isolated hepatocytes

To determine the uptake rate of epirubicin, isolated hepatocytes (1.7×10^6 cells/ml) were incubated at 37 °C with various concentrations of epirubicin. After 5 min of preincubating the cells at 37 °C, an aliquot (50 µl) of epirubicin was added to start uptake. Initial concentrations of epirubicin ranged from 5 to 400 µg/mL. The incubation medium (albumin-free) in the uptake experiment contained 137 mM NaCl, 5.4 mM KCI, 1.25 mM $CaCl_2$, 1.0 mM $MgCl_2$, 0.8 mM $MgSO_4$, 0.5 mM NaH_2PO_4, 0.4 mM Na_2HPO_4, 4.2 mM $NaHCO_3$, 10 mM HEPES, and 5 mM glucose (pH 7.4).

An aliquot of the cell suspension (100 µL) was taken at the indicated times (30, 60, 90, 120, 180, 300 and 600 s), and laid on top of a two-phase system in microfuge tubes. The bottom phase consisted of glycerol (100 µL). This was overlaid with 500 µL of silicone mineral oil (density 1.015). The tubes were then centrifuged for 20 s in a tabletop microfuge (Beckman Instruments, Fullerton, CA, USA). Three milliliters of methanol was added to the glycerol solution. After centrifugation of the mixtures at 3,000 rpm for 15 min at 4 °C, epirubicin amounts in the organic phase were measured as described above. Amounts taken up by hepatocytes were corrected for the adherent film (2.2 µL/mg protein). Adherent water volume was determined with [^{14}C] inulin and $3H_2O$ (Yamazaki et al. 1992). Protein was determined by protein assay kits (Bio-Rad Co. Ltd., Hercules, CA, USA). Bovine serum albumin was the standard.

Determination of the uptake permeability clearance

The initial uptake rate of epirubicin (v_{inf}) was obtained by regression of the linear portion of the uptake time course (within 2 min). The relationship between initial uptake rate (v_{inf}) and the initial epirubicin concentration in the medium (s) was plotted as an Eadie–Hofstee plot. This plot revealed the straight line, and therefore we fitted the uptake data to a Michaelis–Menten equation with a saturable component.

$$v_{\text{inf}} = \frac{\text{Vmax} \cdot [\text{S}]}{\text{Km} + [\text{S}]}$$

where V_{max} and K_m represent the maximum uptake velocity and Michaelis–Menten constant, respectively. The V_{max} and K_m values were calculated with a nonlinear least squares method. Furthermore, we obtained the permeability-surface area product (PS_{inf}) for epirubicin uptake in vitro, which was calculated as V_{max}/K_m after converting the V_{max} value to that per gram of liver, assuming that 1 mg protein contains 1.1×10^6 cells and 1 g liver contains 1.3×10^8 cells (Yamazaki et al. 1992).

Effect of temperature, various inhibitors or substrates on the uptake rate of epirubicin

Temperature dependency of the epirubicin uptake was measured at 0, 27 and 37 °C. The rate of change of a biological system as a consequence of increasing the temperature ($Q_{10,}$ temperature coefficient) was calculated as: $Q_{10} = (v_{inf}$ at 37 °C)/(v_{inf} at 27 °C). To investigate the effects of inhibitors on the initial uptake rate of epirubicin, p-chloromercuribenzenesulfonic acid (PCMB, 500 µM, sulfhydryl-modifying agent), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS, 500 µM, anion exchange inhibitor), rotenone (30 µM, metabolic inhibitor) and carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP, 2 µM, metabolic inhibitor) were tested with the same method of Yamazaki et al. (1993). Various substrates of uptake transporters such as organic anion transporting polypetides (Oatps), organic cation transporters (Octs) and a sodium taurocholate cotransporting polypeptide (Ntcp) were also evaluated: bromosulfophthalein (BSP, 50 µM, Oatp1 and Oatp3 substrate), digoxin (1 µM, Oatp2 substrate), metformin (100 µM, Oct1 substrate), cimetidine (25 µM, Oct2 substrate) and taurocholate (500 µM, Ntcp substrate) (Yamazaki et al. 1993).

Kinetic analysis after i.v. infusion of epirubicin

After epirubicin was infused through the rat femoral vein at a flow rate of 40, 100, 200, 300 or 400 µg/min/kg, the concentrations of epirubicin in the plasma, bile and liver were measured as described above. Biliary clearances based on plasma and liver concentrations of epirubicin ($CL_{bile,plasma}$, $CL_{bile,liver}$) were determined as follows

$$CL_{bile,plasma} = V_{bile}/C_{ss,plasma}$$

$$CL_{bile,liver} = V_{bile}/C_{ss,liver}$$

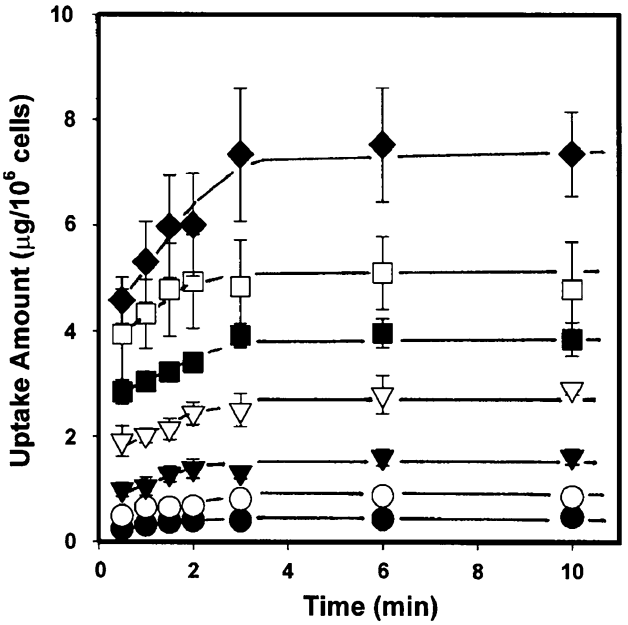

Fig. 1 Time course of uptake of epirubicin into isolated hepatocytes. Hepatocytes (1.7×10^6 cells/ml) were preincubated for 5 min at 37 °C prior to the addition of epirubicin. The initial concentrations were ranged from 5 to 400 μg/mL. Each point represents the mean ± S.E. of three experiments. Keys: (●) 5 μg/mL; (○) 10 μg/mL; (▼) 25 μg/mL; (▽) 50 μg/mL; (■) 100 μg/mL; (□) 200 μM; (◆) 400 μg/mL

where V_{bile} is the biliary excretion rate of epirubicin, and $C_{ss.plasma}$ and $C_{ss.liver}$ represent the plasma and liver concentration of epirubicin, respectively, at steady-state (8 h) after i.v. infusion.

Statistical analysis

The unpaired Student's *t* test was used to compare two groups. One-way analysis of variance was used to test for significant differences between multiple groups. Statistical significance was defined as $P < 0.05$.

Results

Concentration dependency of epirubicin uptake by isolated hepatocytes

Figure 1 shows the time courses of epirubicin uptake by isolated hepatocytes at various concentrations (5–400 μg/mL). The process of epirubicin uptake is linear within 2 min for each concentration, and the initial uptake rate (v_{inf}) for each epirubicin concentration was calculated from the initial slope by linear regression. When the uptake rates were plotted versus the intial epirubicin concentration, a hyperbolic curve was obtained, demonstrating the epirubicin uptake process that increased in a saturated manner (Fig. 2A). A linear transformation of this curve (Eadie-Hofstee plot) yields a straight line (Fig. 2B). The maximal uptake velocity (V_{max}) and the Michaelis–Menten constant (K_m) were calculated to be 3.70 ± 0.21 (μg/min/10^6 cells), and 99.1 ± 6.41 μg/mL, respectively.

Effect of temperature, metabolic inhibitors, sulfhydryl-modifying reagent and anion exchange inhibitor on the initial uptake rate of epirubicin

The epirubicin uptake exhibited remarkable temperature dependency and the initial uptake rate decreased by 57.4 % at 27 °C and 37.3 % at 0 °C (Fig. 3A). The Q10 value was thus calculated as 1.74 (v_{inf} at 37 °C divided by v_{inf} at 27 °C). The uptake decreased greatly after the addition of either a sulfhydryl-modifying reagent (PCMB, 500 μM) or an anion exchange inhibitor (DIDS, 500 μM). PCMB and DIDS inhibited the initial uptake rate of epirubicin by 37.8 and 53.7 % of initial values, respectively. In addition, in the presence of metabolic inhibitors such as rotenone (30 μM) or FCCP (2 μM), initial uptake rate of epirubicin fell to 49.7 and 59.5 % of initial levels, respectively.

Inhibition of initial uptake rate of epirubicin by organic anion transport substrates and organic cation transport substrates

We tested effects of various substrates for organic anion transporters and organic cation transporters on the initial uptake of epirubicin into isolated rat hepatocytes (Fig. 3B). TCA (Ntcp substrate) significantly inhibited the initial uptake rate of epirubicin by 54.8 % of the initial value. Moreover, BSP (Oatp1 and Oatp3 substrate) also significantly inhibited the velocity by 51.4 % of the initial value. In contrast, digoxin (Oatp2 substrate), cimetidine (Oct2 substrate) and metformin (Oct1 substrate) slightly reduced the uptake of epirubicin, but its statistical significance was not determined.

In vivo i.v. infusion studies

After i.v. infusion of epirubicin into rats at flow rates of 10, 20, 40, 80 and 160 μg/min/kg, the plasma concentration and biliary excretion rate were measured. Both parameters reached at steady-state 8 h after i.v. infusion (Fig. 4). We measured the epirubicin concentration at steady-state (8 h) in the plasma ($C_{ss,plasma}$), liver ($C_{ss,liver}$) and bile ($C_{ss,bile}$) after i.v. infusion. The epirubicin concentration ratio at steady-state was obtained by regression of the linear portion of the curve (Fig. 5). The ratios of $C_{ss,liver}/C_{ss,plasma}$, $C_{ss,bile}/C_{ss,liver}$ and $C_{ss,bile}/C_{ss,plasma}$ were calculated to be

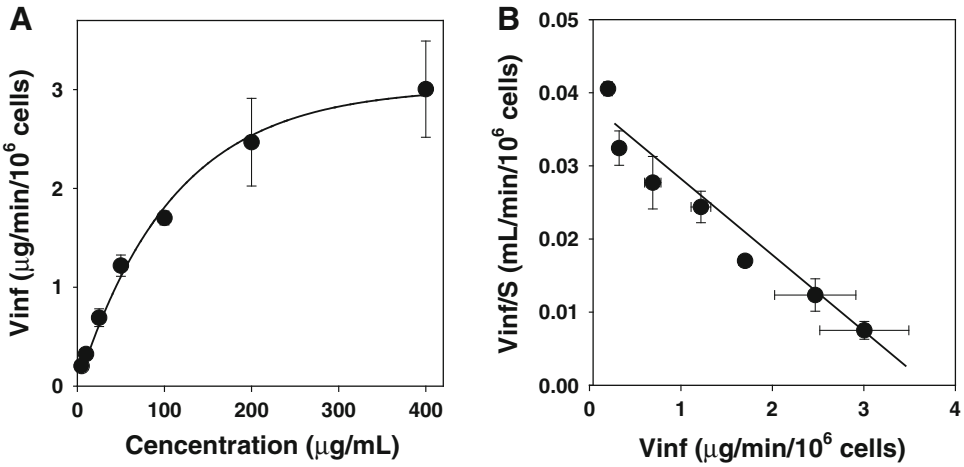

Fig. 2 Kinetics of epirubicin uptake in isolated rat hepatoyctes. (**A**) Plot of initial uptake rate versus epirubicin concentration. (**B**) Eadie-Hofstee plot of epirubicin uptake into isolated hepatocytes. The iInitial uptake rate was measured within 2 min of incubation, and was calculated by linear regression. Each point represents the mean ± SE of three experiments

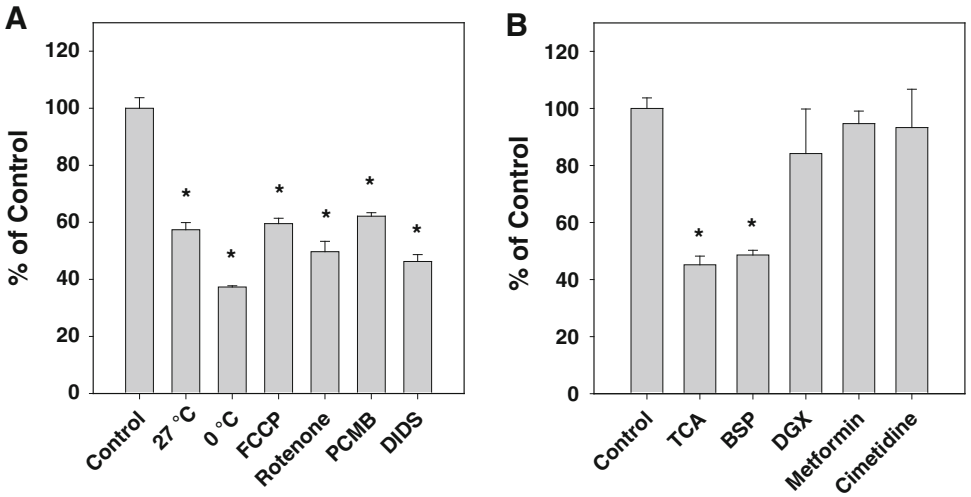

Fig. 3 Effect of temperature, inhibitors or substrates on the initial uptake velocity of epirubicin (15 μg/mL) by isolated hepatocytes. Each *bar* represents the mean ± SE of three experiments. *Significantly different from the control group ($P < 0.01$). (**A**) Temperatures (27 °C, 0 °C), metabolic inhibitors (FCCP, *p*-trifluoromethoxyphenylhydrazone, 2 μM; Rotenone, 30 μM), sulfhydryl-modifying reagent (PCMB, *p*-chloromercuribenzenesulfonic acid, 500 μM) and anion exchange inhibitor (DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid, 500 μM). (**B**) Organic anion transport substrates (TCA, taurocholate, 500 μM; BSP, bromosulfophthalein, 50 μM; DGX, digoxin, 1 μM) and organic cation transport substrates (metformin, 100 μM; cimetidine, 25 μM)

152 ± 9.53, 3.99 ± 0.256 and 513 ± 47.4, respectively. These values indicate that epirubicin accumulated extensively in the liver, followed by excretion into bile. We calculated the biliary excretion clearances based on the plasma ($CL_{bile,plasma}$) and liver ($CL_{bile,liver}$) concentrations of epirubicin. The $CL_{bile,plasma}$ and $CL_{bile,liver}$ decreased as $C_{ss,plasma}$ and $C_{ss,liver}$ increased (Fig. 6). The extent of the $CL_{bile,liver}$ decrease was more pronounced than that of $CL_{bile,plasma}$.

Discussion

Epirubicin, an antineoplastic drug, is widely used in chemotherapy for hepatocelluar carcinoma. Thus, studies on the efflux process of epirubicin have been widely reported in tumor cells and animal tissues (Hu et al. 1999; Lo 2000, 2003). However, accurate kinetic analyses of the cellular uptake of epirubicin have not been reported. Consequently, in the present study, we have characterized the hepatic uptake of epirubicin by isolated hepatocytes. The uptake process of epirubicin was saturable with a K_m of 99.1 μg/mL and V_{max} of 3.70 μg/min/10^6 cells (Fig. 1), which is comparable with Iwakiri's study (2008). They determined the intracellular drug amount 10 min after incubation by the primary cultured hepatocytes. In the present study, we measured the initial uptake velocity of epirubicin within 2 min after incubation by fresh isolated hepatocytes. As shown in the Eadie–Hofstee plot, the uptake process of epirubicin revealed one saturable component (Fig. 2).

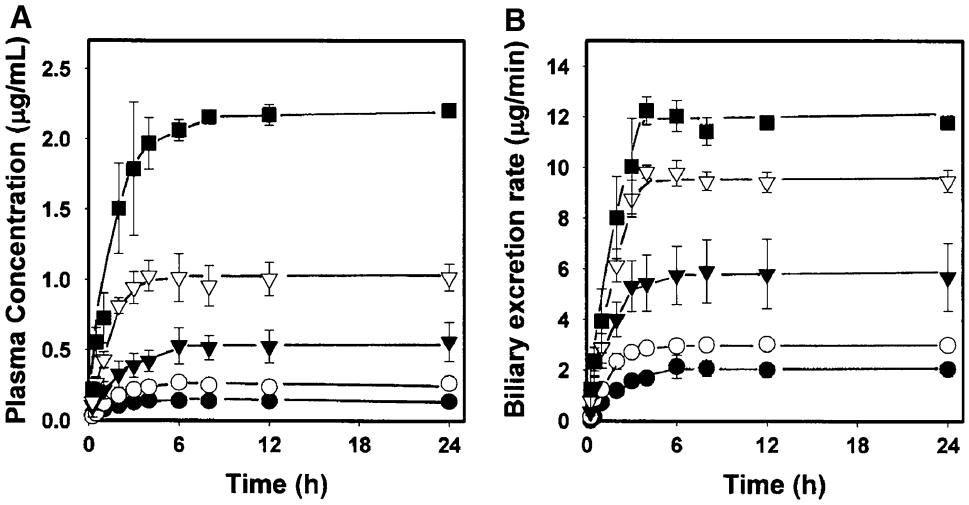

Fig. 4 Plasma concentration (**A**) and biliary excretion rate (**B**) of epirubicin after i.v. infusion in rats. The infusion rates were 10 (●), 20 (○), 40 (▼), 80 (▽) and 160 μg/min/kg (■). Each point represents the mean ± SE of three rats

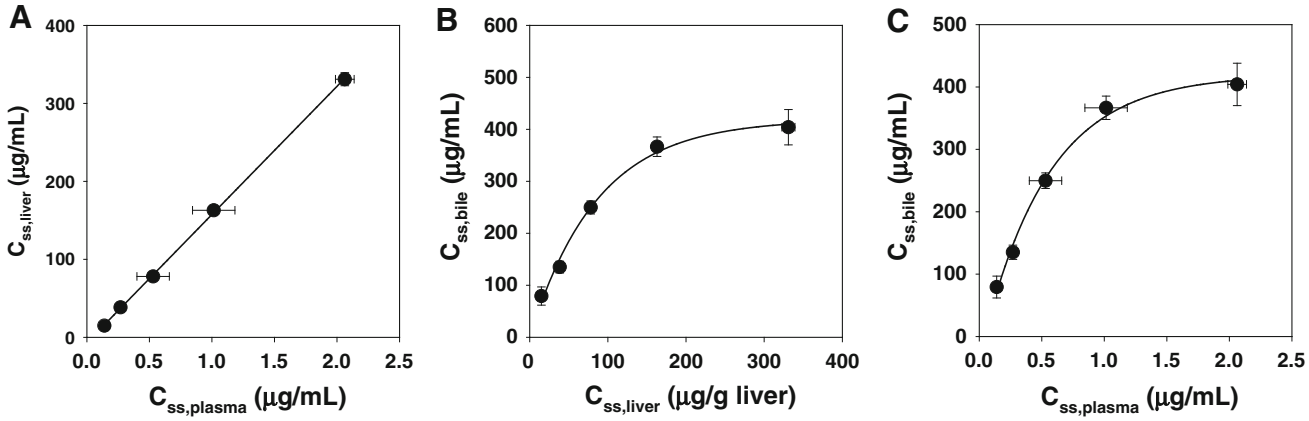

Fig. 5 Plots of $C_{ss,liver}$ vs $C_{ss,plasma}$ (**A**), $C_{ss,bile}$ vs $C_{ss,liver}$ (**B**), and $C_{ss,bile}$ vs $C_{ss,plasma}$ (**C**) of epirubicin after i.v. infusion in rats. The infusion rates were 10, 20, 40, 80 and 160 μg/min/kg. The concentrations of plasma, liver and bile were measured at steady-state after i.v. infusion of epirubicin. Each point represents the mean ± SE of three rats

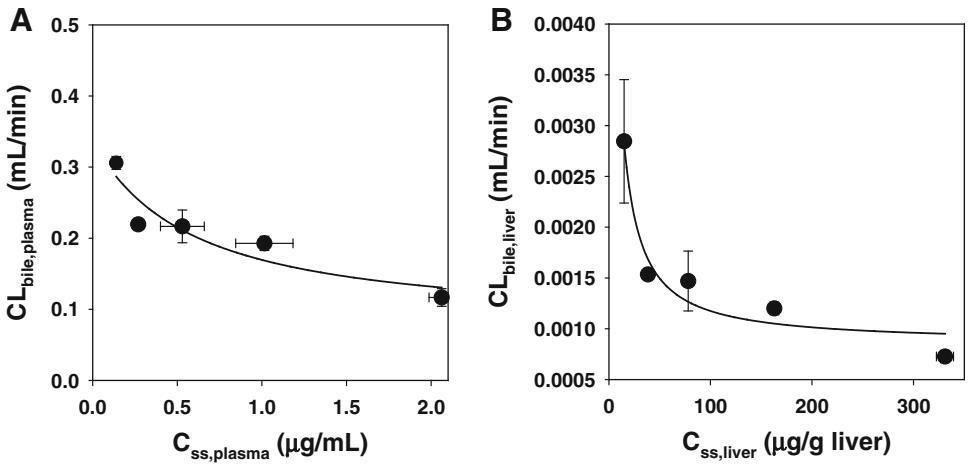

Fig. 6 Biliary excretion clearance based on the plasma (**A**) and liver (**B**) concentrations at steady-state after i.v. infusion of epirubicin in rats. The infusion rates were 10, 20, 40, 80 and 160 μg/min/kg. Each point represents the mean ± SE of three rats

Taking into account the fact that therapeutic serum epirubicin concentration is much lower than the K_m value (Danesi et al. 2002), the carrier-mediated uptake clearance under linear condition (V_{max}/K_m) can be calculated to be 37.4 μL/min/10^6 cells. Based on the reported value, 1 g liver contains 1.3 × 10^8 cells (Yamazaki et al. 1992), the

PS_{inf} value is calculated as 4.86 mL/min/g liver. These analyses suggest that epirubicin might be taken up by hepatocytes via a carrier-mediated system.

The initial uptake velocity of epirubicin was significantly inhibited in a temperature-dependent manner (Fig. 3A), suggesting that epirubicin might depend on energy to be taken up by hepatocytes. Indeed, the epirubicin influx process was substantially inhibited by ATP suppression in hepatocytes. The initial uptake velocity of epirubicin by the hepatocytes was significantly inhibited in the presence of metabolic inhibitors such as rotenone or FCCP (Fig. 3A). Yamazaki et al. (1993) have demonstrated that metabolic inhibitors (rotenone and FCCP) decreased the cellular ATP level rapidly within 1 min to 19 and 15 % of the initial value, respectively. However, 30 or 15 % of the initial value was still maintained after a 30-min incubation with rotenone and FCCP, respectively. In the present study, the initial uptake velocity of epirubicin remained at 46–63 % of the control value (Fig. 3A). The uptake velocity of epirubicin was also significantly reduced after the addition of sulfhydryl-modifying reagent (PCMB) or an anion exchange inhibitor (DIDS) (Fig. 3A). These findings indicated that the uptake of epirubicin might be mediated by the active transport protein in hepatocytes.

Substrates for organic anion transporters such as TCA and BSP significantly inhibited the initial uptake velocity of epirubicin (Fig. 3B). In contrast, statistical significance for epirubicin uptake was not determined after the addition of substrates for organic cation transporters such as cimetidine and metformin (Fig. 3B). Iwakiri's study (2008) with the cultured hepatocytes demonstrated that the uptake process of epirubicin revealed biphasic profiles, and that the process was slightly reduced by the treatment of organic cation transport inhibitors such as verapamil and tetraethylammonium. However they measured the total intracelluar amount of drug at one time period at the end of the incubation. In the present study, the initial uptake rate (v_{inf}) for each epirubicin concentration was calculated from the initial slope by a linear regression. It is likely that in their study, a one-point determination of the amount of drug taken up was not enough to evaluate the initial uptake velocity by the hepatocytes. However, the major results of Iwakiri's study (2008) are comparable to the present study, and suggest that inhibiting the transporter involved in the cellular influx of epirubicin mitigated its cytotoxicity. Taken together, the two criteria for carrier-mediated active transport were satisfied in the hepatic transport of epirubicin; one is that metabolic energy is required for epirubicin transport, and the other is that epirubicin was taken up via organic anion transporting polypeptides.

We also attempted to determine the hepatobiliary transport of epirubicin after in vivo i.v. infusion. In our previous study (Shin et al. 2013), epirubicin accumulated selectively into the liver after an i.v. bolus administration in rats, and was mostly excreted into bile. The concentrating process in biliary excretion from the liver might be one reason for the selective accumulation of epirubicin in bile. However, this potential mechanism has not been clarified yet. In the present study, at the steady-state after i.v. infusion of epirubicin, $C_{ss,liver}$ was about 152-fold higher than $C_{ss,plasma}$, and $C_{ss,bile}$ was about 3.99-fold higher than $C_{ss,liver}$ in the linear portion of the curve (Fig. 5), indicating that epirubicin extensively accumulated in the liver, followed by excretion into bile. These findings suggest that both hepatic uptake and biliary excretion of epirubicin are mediated via specific transporters. Recently, we clarified the dose-dependent pharmacokinetics of epirubicin after its i.v. bolus administration in rats (Shin et al. 2013). In the previous study, the CL_{tot} values significantly decreased after i.v. administration with the increase in dose from 2 to 50 mg/kg. The amounts of epirubicin found in the bile by 12 h after its administration of 2–50 mg/kg decreased from 27.9 to 21.5 % of the initial dose, however, the significant differences were not observed (Shin et al. 2013). Unlike the single i.v. bolus administration study (Shin et al. 2013), the $CL_{bile,plasma}$ and $CL_{bile,liver}$ decreased as $C_{ss,plasma}$ and $C_{ss,liver}$ increased at the steady-state after i.v. infusion of epirubicin (Fig. 6). The extent of the $CL_{bile,liver}$ decrease was more pronounced than that of $CL_{bile,plasma}$ (Fig. 6), suggesting that its saturation in hepatobiliary transport might be mainly responsible for the nonlinearity of the biliary excretion process. Recently, it has been demonstrated that the efflux of the lipophilic antineoplastic drugs from tumor cells were extensively stimulated via transport proteins such as P-glycoprotein (P-gp) and multidrug-resistance associated proteins (MRP) (Nagasawa et al. 1996; Hu et al. 1999; Lo 2000). Therefore, these proteins may play a major role in the biliary excretion of epirubicin from the bile canalicular membrane. In conclusion, present studies using isolated rat hepatocytes and in vivo i.v. infusion demonstrate that epirubicin is likely to be taken into the liver cells via organic anion transporting polypeptides, and that its biliary excretion might be mediated via specific transporters.

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