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（ラットにおける肝臓の P 糖たん白質および多剤耐性関連たん白（Mrp2）の発現と機能の性差）

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TATSUYA SUZUKI

Abstract

To clarify whether gender-related differences exist in the expression and function of hepatic P-glycoprotein- and/or multidrug resistance-associated protein (Mrp2), we measured the hepatobiliary excretion of doxorubicin, and their protein levels in male and female Sprague-Dawley rats. When rats received a single intravenous injection of doxorubicin (5 mg/kg), a delay in the disappearance of doxorubicin from plasma was observed in male rats. When rats received a constant-rate infusion of doxorubicin, no significant gender-related differences in the apparent biliary clearance of doxorubicin based on the steady-state plasma concentrations were observed between male and female rats. However, the net biliary clearance of doxorubicin based on the liver concentration, which represents the actual function of P-glycoprotein and/or Mrp2, was higher in female rats than in male rats. These results suggest that the actual function of the hepatobiliary transport of doxorubicin is greater in female than in male rats. Western blot analysis revealed that the expression of P-glycoprotein and Mrp2 in the liver of female rats was significantly higher

than in male rats, similar to results of hepatobiliary excretion experiments. The expression of hepatic cytochrome P450 (CYP) 2B1, which is involved in the metabolism of doxorubicin, was significantly higher in male than in female rats. By pretreatment with testosterone (10 mg/day for 7 days), the actual biliary clearance of doxorubicin in female rats was nearly that of male rats. The protein levels of P-glycoprotein and Mrp2 in female rats were also lowered by treatment with testosterone so as to be nearer those in male rats. These results suggest that gender-related differences exist in P-glycoprotein- and Mrp2-mediated hepatobiliary transport and that these two transporters may be regulated by sex hormones.

Introduction

P-glycoprotein, a member of the ATP-binding cassette (ABC) transport proteins, is known to efflux hydrophobic and cationic antitumor drugs such as anthracycline antibiotics and *Vinca* alkaloids from anticancer drug-resistant cells. This drug transporting protein is present not only in anticancer drug-resistant cells, but also in a variety of normal tissues, including the canalicular membrane of hepatocytes, the brush border membrane of renal proximal tubule cells and small intestinal epithelial cells. It acts as an efflux transporter protein or has a protective function for endogenous and exogenous toxic substances (Thiebaut et al., 1987; Cordon-Cardo et al., 1990; Schinkel et al., 1994). Like P-glycoprotein, multidrug resistance-associated protein 2 (Mrp2) is present in almost the same tissues as P-glycoprotein (Borst et al., 1999; König et al., 1999). This transporter also acts as an active efflux pump for a wide range of organic anions such as glucuronide and sulfate conjugates, through an ATP-dependent mechanism (Oude Elferink et al., 1995).

Thus, both drug transporters appear to play key roles in the hepatobiliary excretion of a variety of drugs and their metabolites as well as hepatic cytochrome P450 (CYP) enzymes.

Our interest in gender-related differences in the expression of P-glycoprotein and/or Mrp2 arose from those reports suggesting the presence of gender-related differences in hepatic P-glycoprotein expression in humans and rats (Schuetz et al., 1995; Salphati and Benet, 1998). Gender-related differences in mammalian drug pharmacokinetics are one of major determinants for the clinical effectiveness of drug therapy and are mostly caused by differences in the expression of hepatic CYP isoforms and drug transporting proteins (Harris et al., 1995; Meibohm et al., 2002). Schuetz et al. (1995) reported that men have a significantly higher level of P-glycoprotein compared with women. In contrast, Salphati and Benet (1998) reported that the protein level of P-glycoprotein is higher in female rats than in male rats. Although numerous substrates for P-glycoprotein, Mrp2 and CYP3A largely overlap (Mayer et al., 1995; Oude Elferink et al., 1995; Wacher et al., 1995), gender-related differences in the protein level and activity of CYP3A4 are not observed in

humans (Schmucker et al., 1990; George et al., 1995; Meibohm et al., 2002). Therefore, gender-related differences in mammalian hepatobiliary excretion might be due to differences in the expression of P-glycoprotein and/or Mrp2, but not CYP3A.

Doxorubicin, an anthracycline anticancer drug known to be a substrate of P-glycoprotein, is primarily excreted into bile. This drug is also a potential substrate for multidrug resistance-associated protein (Mrp2) (Cui et al., 1999), and is primarily excreted into the bile via P-glycoprotein and Mrp2 (Speeg and Maldonado, 1994; van Asperen et al., 2000; Hidemura et al., 2003). It is reported that CYP2B1 is the principal isoform responsible for the hepatic metabolism of doxorubicin in rats (Goeptar et al., 1993), though there is no information available regarding gender-related differences in the expression of CYP2B1 in humans and animals. On the basis of these observations, doxorubicin is considered as a model drug for clarifying the effects of gender on the expression and function of hepatic P-glycoprotein and/or Mrp2 in rats. We have recently demonstrated that the down-regulation of hepatic P-glycoprotein and Mrp2 in the rat liver directly leads

to a reduction in P-glycoprotein- and Mrp2-mediated hepatobiliary transport function of doxorubicin (Hidemura et al., 2003). The investigation of the mechanisms responsible for gender-related differences in hepatobiliary transport function of doxorubicin in rats may help in the interpretation of pharmacokinetic and pharmacodynamic data obtained from humans, because rats are used in many studies in order to develop pharmacokinetic models that could be easily scaled-up to humans.

The purpose of the present study was to clarify whether gender-related differences exist in the expression of hepatic P-glycoprotein, Mrp2 and CYP2B1, which are related to the pharmacokinetic behavior of doxorubicin in rats. The effect of gender on the function of P-glycoprotein- and/or Mrp2-mediated hepatobiliary transport of doxorubicin was also investigated.

Materials and methods

1. Chemicals

Doxorubicin hydrochloride and daunorubicin hydrochloride were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). Doxorubicin hydrochloride, in the form of a commercial preparation for injection used in the *in vivo* experiment, was purchased from Kyowa Hakko Kogyo (Tokyo, Japan). All reagents used in this study were used without further purification. Testosterone was purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents are commercially available and were of analytical grade.

2. Animals

Sprague-Dawley (SD) age-matched (10-week-old) female and male rats (SLC Japan, Hamamatsu, Japan), weighing approximately 250 and 310 g, respectively, were used in this

study. The animals were housed under controlled environmental conditions (temperature 23 ± 1^{o}C and humidity 55 ± 5%) with a commercial food and water freely available. The procedures involving animals and their care conformed to the international guidelines, Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985) and Guiding Principles for the Care and Use of Laboratory Animals of Nagoya University, Japan.

3. Pharmacokinetic study

To determine the pharmacokinetics of doxorubicin, rats were anesthetized with pentobarbital (40 mg/kg of body weight), and the right jugular vein of each rat was cannulated with a polyethylene tube for drug administration and blood sampling. The rats received a single intravenous injection of doxorubicin (5 mg/kg). Blood samples were collected from the jugular vein in freely moving rats at 3, 6, 10, 20, 30, 40, 60, and 90 min after drug administration. After each blood sampling the jugular vein cannula was flushed

with saline (~ 0.25 ml). Plasma was immediately separated by centrifugation at 3,800 × g for 10 min at 4 ^{o}C and stored at -30 ^{o}C until analysis.

To perform biliary clearance experiments, the rats under light anesthesia with pentobarbital (40 mg/kg) were cannulated with polyethylene tubing into the left jugular artery and the bile duct for the collection of blood and bile samples, respectively. The rats received a bolus intravenous injection of doxorubicin in loading doses of 0.80 mg/kg for male rats and 0.36 mg/kg for female rats followed by a constant-rate infusion, using Harvard infusion pump (PHD 2000, South Natick, MA, USA), of saline solution delivering doses of 608 μg/kg/h for male rats and 615 μg/kg/h for female rats of doxorubicin at a rate of 4 ml/h until the end of the study. After 60-min infusion, the steady-state plasma concentration of doxorubicin (approximately 0.15 μg/ml in male and female rats) was attained, and bile was collected in preweighed tubes at 20-min intervals for 60 min throughout the experiment. Blood samples were taken at the midpoints of the bile collection periods (70, 90 and 110 min after the infusion was started). After 120-min

infusion, the rats were killed by severing the main artery and the liver was washed out with ice cold saline. Plasma samples were obtained by the method described above. The volume of bile samples was measured gravimetrically with specific gravity assumed to be 1.0. Plasma, bile and liver were stored at –30 ^{o}C until analysis. This experiment was done under pentobarbital anesthesia, and body temperatures of the animals were maintained at 37 ^{o}C with a heat lamp.

4. Drug analysis

Concentrations of doxorubicin in plasma, bile and liver were determined by high-performance liquid chromatography (HPLC) according to a method reported previously (Hidemura et al., 2003). Bile samples were properly diluted in phosphate-buffered saline (PBS) solution (pH 7.4), and liver was added to four volumes of PBS and homogenized. The liver homogenate was further diluted with two volumes of PBS. Briefly, 20 μl of each sample and 120 μl of mixed solution of acetonitrile containing an

internal standard of daunorubicin (0.2 μg/ml) were mixed and centrifuged at 6,000 × g for 10 min. After centrifugation, the supernatant (100 μl) was subjected directly to HPLC. The apparatus used for HPLC was a Shimadzu LC-10A system (Kyoto, Japan) equipped with a fluorescence detector (RF-10AXL, Shimadzu) (excitation, 475 nm; emission, 580 nm) consisting of an LC-10A liquid pump and an SIL-10A autoinjector. The conditions were as follows: column, a Cosmocil $5C_{18}$ column (4.6 by 150 mm, Nacalai Tesque, Kyoto, Japan); mobile phase, 10 mM phosphate buffer (pH 4.0)-methanol (40 : 60 [vol/vol]) solution; column temperature (CTO-10AC, Shimadzu), 50 ^{o}C; flow rate, 1.2 ml/min. This assay was shown to be linear for the concentrations studied with a correlation coefficient of 0.999. No interference with the peak of doxorubicin was observed in any samples. The detection limit was approximately 0.02 μg/ml for plasma and bile samples and 0.05 μg/g tissue for liver homogenate. The within-day and between-day coefficients of variation for this assay were less than 8%.

5. Data analysis

Plasma concentration-time data for doxorubicin after a single intravenous administration were analyzed using a noncompartmental model. The area under the curve (AUC) and the area under the first moment curve (AUMC) were calculated by the trapezoidal rule with extrapolation to infinity. The systemic clearance (CL_{SYS}) was determined as dose/AUC. The mean residence time (MRT) was calculated as AUMC/AUC. The volume of distribution at steady state was calculated as $CL_{SYS} \times MRT$. Each parameter was calculated with the nonlinear least-squares regression program Winnonlin.

For biliary clearance experiment, the apparent biliary clearance ($CL_{BILE/PLASMA}$) based on plasma concentration was calculated by dividing the biliary excretion rates by C_{SS} determined for that collection period. The net biliary clearance ($CL_{BILE/LIVER}$) based on liver concentration was calculated by dividing the biliary excretion rate by the liver concentration (C_L) represented as the tissue concentration at 120 min after starting infusion.

Each parameter was calculated using the mean value for three datum points during 60 min. The K_p value is represented as the ratio of C_L to C_{SS}.

6. Hepatic microsome preparation

Male and female rats under light pentobarbital anesthesia (40 mg/kg) were killed by exsanguinations. The liver was excised after the perfusion of a sufficient volume of ice cold saline to remove most of the blood. Microsomal samples were prepared from liver homogenized samples of male and female rats as previously described (Omura and Sato, 1964). Briefly, liver (approximately 0.2 g) was homogenized in 1.15% KCl solution at 4 ^{o}C with a Teflon homogenizer. The homogenate was centrifuged at 12,000 × g for 25 min at 4 ^{o}C. The supernatant was further centrifuged at 100,000 × g for 90 min at 4 ^{o}C to obtain the microsomal fraction. The obtained pellet was resuspended in 1.15% KCl and homogenized with a loose daunce homogenizer, and then the suspension was ultracentrifuged again. The protein concentration of the microsomal samples was

measured with Bio-Rad Protein Assay (Bio-Rad Laboratories, Richmond, CA, USA) using bovine serum albumin (Sigma Chemicals) as a standard. The samples were kept at -80 ^{o}C until analysis.

7. Western blot analysis

Western blot analysis of CYP2B1 was performed according to methods reported previously (Kitaichi et al., 2004). Briefly, the protein (2 μg) was separated by electrophoresis on 10% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) and transferred to nitorocellulose membrane. The membrane was blocked in PBS solution containing 0.1% Tween 20 and 5% nonfat dry milk, detected by goat monoclonal antibody to rat CYP2B1 (Daichi Pure Chemicals, Tokyo, Japan). The immune complexes were visualized using horseradish peroxidase-labeled secondary antibody with ECL Western blot detection reagents (Amersham Biosciences Co.).

Western blot analysis of P-glycoprotein and Mrp2 was performed according to

methods reported previously (Hidemura et al., 2003). Briefly, each liver excised after washing with a sufficient volume of ice cold saline was suspended in 10-fold volumes of 10 mM Tris-HCl buffer (pH 8.0) containing, 1.5 μg/ml of aprotinin and 1 mM phenylmethylsulfonyl fluoride (Sigma Chemicals). The suspension was homogenized with a tight homogenizer (10 strokes up and down) and centrifuged at 3,000 × g for 10 min at 4 ^{o}C. The pellet was dissolved in 100 μl of 10 mM Tris-HCl buffer containing 0.5 μl of Nonidet P-40.

The protein (20 μg) was separated by electrophoresis on 8% polyacrylamide gel containing 0.1% sodium dodecyl sulfate (SDS) and transferred to a nitrocellulose membrane. The membrane was blocked in PBS containing 0.1% Tween 20 and 5% nonfat dry milk, detected by C219 mouse monoclonal antibody to P-glycoprotein (DAKO A/S, Glostrup, Denmark) and human monoclonal antibody to Mrp2 (Alexis, Biochemicals, San Diego, CA, USA).

To quantify the relative levels of P-glycoprotein, Mrp2, and CYP2B1 in each

membrane, the intensity of the stained bands was measured by the NIH image program (Bethesda, MD, USA).

8. Statistical analysis

Results were expressed as means ± S.E.M. for the indicated number of experiments. Statistical comparisons between groups were assessed by Student's *t*-test or one-way analysis of variance (ANOVA). When *F* ratios were significant ($P<0.05$), Scheffe's post hoc tests between two groups were done, and *P* values less than 0.05 were considered statistically significant differences.

Results

1. Effect of gender on the pharmacokinetics of doxorubicin

We first compared the plasma concentration-time curves of doxorubicin in male and female rats. Fig. 1 shows the mean semilogarithmic plots of plasma concentration-time data for doxorubicin after a single intravenous injection of doxorubicin (5 mg/kg) in male and female rats. As shown in Fig. 1, the plasma concentrations of doxorubicin at 40 and 60 min after injection in male rats were significantly higher than those in female rats. A slight, but not significant, delay in the disappearance of doxorubicin from plasma was observed in male rats.

In the single pharmacokinetic study, it was found that the elimination of doxorubicin, which is mainly excreted into the bile, tended to be delayed in male rats. To investigate whether gender-related differences exist in the hepatobiliary excretion of doxorubicin, we carried out hepatobiliary clearance experiments. The biliary clearance parameters of

doxorubicin in male and female rats are summarized in Table 1. No significant differences were observed between male and female rats in the plasma concentrations of doxorubicin at steady-state (0.16 ± 0.02 and 0.14 ± 0.01 μg/ml) and apparent biliary clearance ($CL_{BILE/PLASMA}$) based on the plasma concentrations (26.4 ± 3.99 and 25.0 ± 1.31 ml/min/kg). However, the net biliary clearance ($CL_{BILE/LIVER}$) of doxorubicin based on the liver concentration of doxorubicin, which represents the actual hepatobiliary transport function of doxorubicin, was larger in female than in male rats (2.11 ± 0.28 and 0.99 ± 0.15 g/min/kg), indicating that the actual function of P-glycoprotein and/or Mrp2 in the liver was potentially reduced in male rats. The concentration of doxorubicin in liver and the liver-to-plasma concentration ratio (K_P) were higher in male rats than in female rats (4.15 ± 0.34 and 1.77 ± 0.13 μg/g, 29.0 ± 2.94 and 12.8 ± 1.10). We further investigated the effect of testosterone on the hepatobiliary excretion of doxorubicin in female rats. The net biliary clearance of doxorubicin in female rats approximated that in male rats (2.11 ± 0.28 and 0.64 ± 0.08 g/min/kg).

2. Western blot analysis of P-glycoprotein and Mrp2 levels in liver

It was found that gender-related differences exist in the hepatobiliary transport of doxorubicin. To clarify whether gender-related differences exist in the expression of P-glycoprotein and Mrp2 in liver, we measured the liver protein levels of P-glycoprotein and Mrp2 by Western blot analysis. The constitutive expression of P-glycoprotein and Mrp2 in liver of male rats was significantly lower than that in female rats (Figs. 2 and 3). To investigate whether sex hormones are involved in the regulation of these two transporters, we further investigated the effect of testosterone on the expression of P-glycoprotein and Mrp2. The protein levels of P-glycoprotein and Mrp2 in the liver of female rats pretreated with testosterone approximated that in male rats.

3. Effect of gender on expression of CYP2B1 in liver

We examined the effect of gender on the protein levels of CYP2B1 in liver by Western blot analysis. The constitutive hepatic expression of CYP2B1 in female rats was

significantly, but slightly, lower than that in male rats (Fig. 4).

Discussion

It is well known that physiological differences between male and female exist in a variety of tissues. Very recently, sex-dependent expression and activity of breast cancer resistance protein (BCRP), a member of the ABC transporter family, in the mouse liver has been reported (Merino et al., 2005). However, it remains unclear whether gender-related differences exist in the expression and function of hepatic drug transporters, P-glycoprotein and Mrp2, in rats, thus far. In the present study, therefore, we measured the hepatobiliary transport of doxorubicin in male and female rats to clarify the gender differences in the function of P-glycoprotein and Mrp2 in the liver, and the expression levels of the transporters were quantified.

We first determined the plasma concentration curves of doxorubicin after a single intravenous injection to male and female rats. The plasma concentrations in male rats were higher that those in female rats at 40 and 60 min during the elimination phase,

suggesting the possibility that hepatobiliary excretion of doxorubicin was delayed in the male rats. To confirm the gender difference in the excretion of doxorubicin, we performed hepatobiliary clearance experiments with the continuous infusion. No significant difference in the apparent biliary clearance based on plasma concentration at steady state ($CL_{BILE/PLASMA}$) was observed between male and female rats. As P-glycoprotein and Mrp2 are located on the bile canalicular membrane of hepatocytes, the strict functional analysis should be based on the liver concentration, not that of plasma. Therefore, the net biliary clearance ($CL_{BILE/LIVER}$) of doxorubicin was calculated, resulting in 0.99 ± 0.15 and 2.11 ± 0.28 g/min/kg in male and female rats, respectively, showing a statistically significant difference. These results suggest that there is a gender difference in doxorubicin transport ability in hepatobiliary excretion. An 80% decrease in hepatobiliary excretion of doxorubicin was observed in *mdr1a*-deficient mice compared with wild mice (van Asperen et al., 2000), implying that the hepatobiliary excretion of doxorubicin is mediated by P-glycoprotein. In addition, MRP2 is reported to be associated with resistance to

doxorubicin in MRP2-expressing cells (Koike et al., 1997; Cui et al., 1999), and MRP2 plays an important role in hepatobiliary excretion of various compounds including conjugated-drugs. Considering that doxorubicin is substrates for both P-glycoprotein and Mrp2 (Speeg and Maldonado, 1994; van Asperen et al., 2000; Hidemura et al., 2003), it is reasonable to speculate that the gender difference in the hepatobiliary excretion of doxorubicin observed in this study may be due to the difference in function of P-glycoprotein and/or Mrp2. Because the expression level of the transporter determinates its function to a great degree, we therefore quantified the expression levels of P-glycoprotein and Mrp2 protein in the liver by Western blot analysis.

In the present study, the expression levels of hepatic P-glycoprotein and Mrp2 were 50% higher in female rats than those in male rats. With respect to the difference of P-glycoprotein expression levels between male and female rats, our results agreed well with the previous report showing higher levels of total *mdr* gene products (mRNA) in female rat livers compared with male livers (Piquette-Miller et al., 1998). Johnson et al. (2002)

measured the expression levels of hepatic Mrp2 in male and female rats between 0 and 90 days of age, and showed that expression levels of Mrp2 protein were higher in female rats than in male rats at the age of 45 days. We also found the higher Mrp2 expression in liver of female rats than that of male rats. Collectively, the higher biliary clearance of doxorubicin in female rats is due, in part, to the functional up-regulation of hepatic P-glycoprotein and Mrp2, which is based on higher expressions of these transporters.

BCRP is expressed in various organs including liver in rodents. Most recently, two research groups have reported higher expression of BCRP in the liver of male mice than female mice (Tanaka et al., 2005; Merino et al., 2005). Tanaka et al. (2005) suggested that the male predominant expression of Bcrp mRNA is caused by the effect of testosterone. Imai et al. (2005) demonstrated that BCRP was down-regulated by estradiol *in vitro*. Although the difference in expression by gender is regulated in an opposite manner, sex hormones might affect the expression of P-glycoprotein and Mrp2. Therefore, the effect of a male-sex hormone testosterone was examined in female rats. After 7-consecutive

daily administrations of testosterone, the protein levels of P-glycoprotein and Mrp2 in livers of female rats were increased compared with normal female rats, and approximated those in male rats, suggesting that testosterone may play a role in regulating the expression of these transporters. Although the precise mechanisms underlying the regulation of P-glycoprotein and Mrp2 expression by testosterone remain unclear, our data suggest that sex hormone may be a regulatory factor that determines the gender difference in hepatobiliary excretion of doxorubicin, although other factors might also be involved in this process.

Doxorubicin is mainly metabolized by CYP2B1 in rats (Goeptar et al., 1993). In the present study, the expression levels of CYP2B1 in female rats were significantly, but slightly, lower than those in male rats. This result is supported by an earlier investigation (Agrawal and Shapiro, 1996), which reported higher hepatic CYP2B1 mRNA levels in male rats compared with female rats. However, the K_p value and liver concentration of doxorubicin in male rats were approximately 2-fold higher than those in female rats.

Given that the expression levels of hepatic CYP2B1 are very low in male and female rats (Yamazoe et al., 1987), the effect of metabolism by CYP2B1 could be minimal. The higher K_p value for doxorubicin observed in male rats may be due, in part, to lower hepatobiliary excretion of doxorubicin. Other factors remaining to be investigated include the lower efflux of doxorubicin into blood from hepatocytes and/or higher uptake of doxorubicin into hepatocytes across the sinusoidal membrane.

Hepatobiliary excretion consists of 2 steps: (1) uptake from the sinusoidal side into the hepatocytes, and (2) efflux from the inside of hepatocytes out into the bile across the canalicular membrane. P-glycoprotein and Mrp2 are expressed in the canalicular membrane and are responsible for the second step. The gender difference in the pharmacokinetics might be smaller than the expected difference estimated from the differences in the levels of P-glycoprotein and Mrp2 expression. The resistance of cancer cells reportedly results not only from the enhancement of the efflux function but also from the reduction of the influx function (Shen et al., 2000). Okabe et al. (2005) have cloned a

novel organic cation transporter which is responsible for the uptake of doxorubicin. Although the transporter is not expressed in normal human liver, there might be other unidentified transporter(s) responsible for the uptake of doxorubicin into hepatocytes, leading to a smaller influence on the pharmacokinetics of drugs than the gender difference in the expression levels of efflux transporters.

In conclusion, the present study demonstrates that gender-related differences exist in the hepatobiliary excretion of doxorubicin in rats. This gender-related difference results, in part, from differences in the expression levels and function of hepatic drug transporters P-glycoprotein and Mrp2, which may be regulated by sex hormones.

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Figure Legends

Fig. 1. Mean semilogarithmic plots of plasma concentration-time data for doxorubicin in male and female rats. Each symbol represents the mean ± S.E.M. (n = 4). Symbols: (●), male rats; (○), female rats. "a" indicates values significantly different from those for male rats ($P < 0.05$).

Fig. 2. Expression of hepatic P-glycoprotein in male and female rats and effect of testosterone on P-glycoprotein and Mrp2 expression in female rats. Each column shows the intensity ratio compared to the value for the male rats. Each value represents the mean ± S.E.M. of four animals. "a" indicates values significantly different from those for male rats ($P < 0.05$).

Fig. 3. Expression of hepatic Mrp2 in male and female rats and effect of testosterone on

P-glycoprotein and Mrp2 expression in female rats. Each column shows the intensity ratio compared to the value for the male rats. Each value represents the mean ± S.E.M. of four animals. “a” indicates values significantly different from those for male rats ($P < 0.05$).

Fig. 4. Expression of hepatic CYP2B1 in male and female rats. Each column shows the intensity ratio compared to the value for male rats. Each value represents the mean ± S.E.M. of four animals. “a” indicates values significantly different from those for male rats ($P < 0.05$).

Table 1. Effect of gender on hepatobiliary excretion of doxorubicin

Parameter	Male	Female	
		Saline	Testosterone
Body weight (g)	342.4 ± 2.4	208.2 ± 2.3[a]	213.7 ± 2.3 [a]
C_{SS} (μg/ml)	0.16 ± 0.02	0.14 ± 0.01	0.16 ± 0.01
$CL_{BILE/PLASMA}$ (ml/min/kg)	26.4 ± 3.99	25.0 ± 1.31	20.0 ± 2.62
$CL_{BILE/LIVER}$ (g/min/kg)	0.99 ± 0.15	2.11 ± 0.28[a]	0.64 ± 0.08 [a,b]
Concentration in liver (μg/g)	4.15 ± 0.34	1.77 ± 0.13[a]	4.56 ± 0.46 [b]
K_P	29.0 ± 2.94	12.8 ± 1.10[a]	29.3 ± 3.77

Each value represents the mean ± S.E.M. (n = 4 to 6).

C_{SS} is steady state plasma concentration of doxorubicin.

$CL_{BILE/PLASMA}$ and $CL_{BILE/LIVER}$ represent biliary clearance based on C_{SS} and liver concentration of doxorubicin, respectively.

K_P represents liver-to-plasma concentration ratio

[a,b] Significantly different from male and female treated with saline, respectively ($P < 0.05$).

Fig. 1.

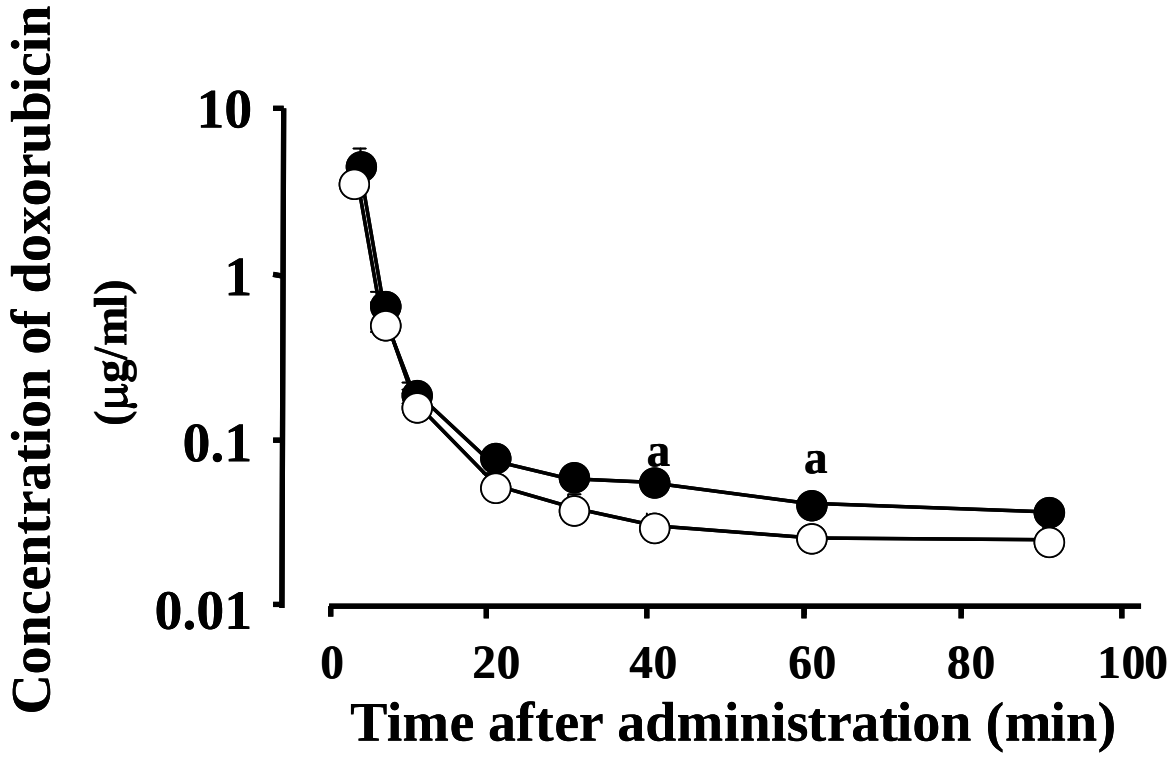
Concentration of doxorubicin
(μg/ml)
10
1
0.1
0.01
a
a
0
20
40
60
80
100
Time after administration (min)

Fig. 2.

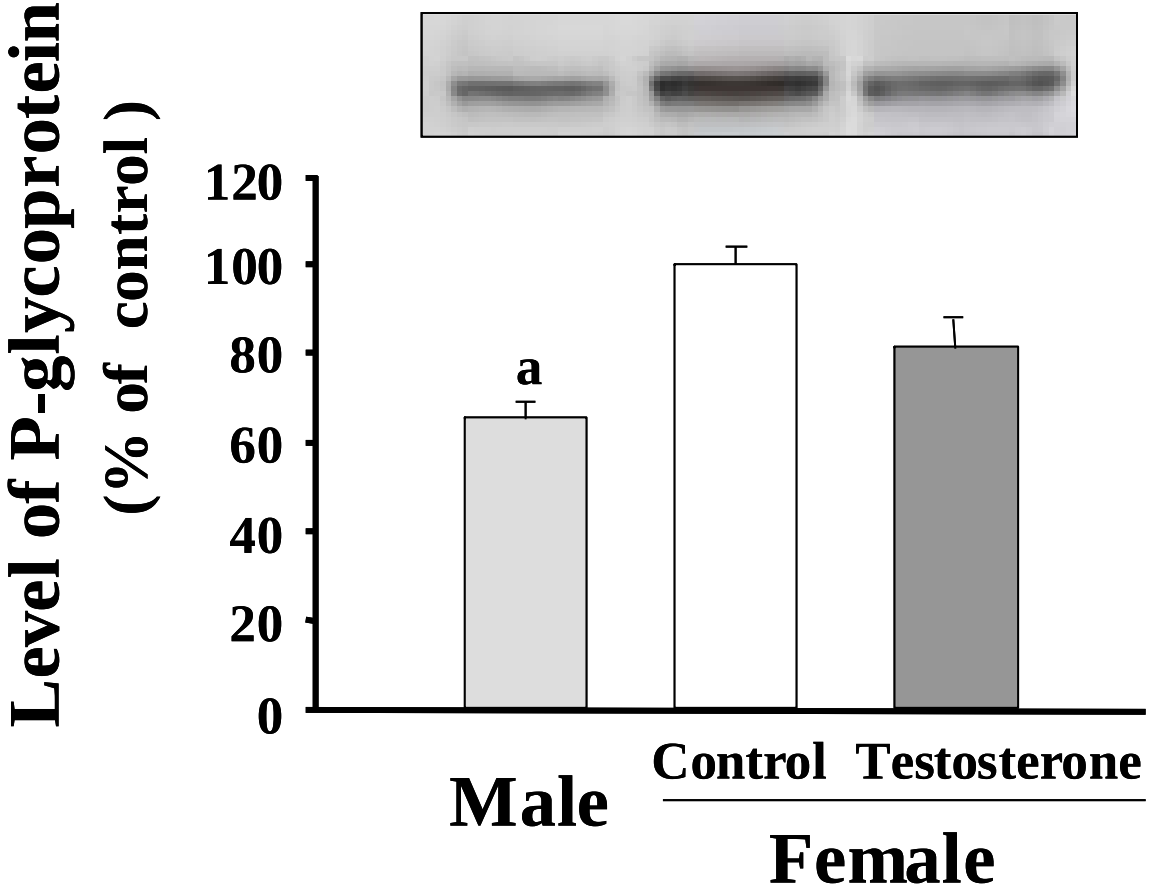
Level of P-glycoprotein
(% of control)
120
100
80
60
40
20
0
a
Male
Control
Testosterone
Female

Fig. 3.

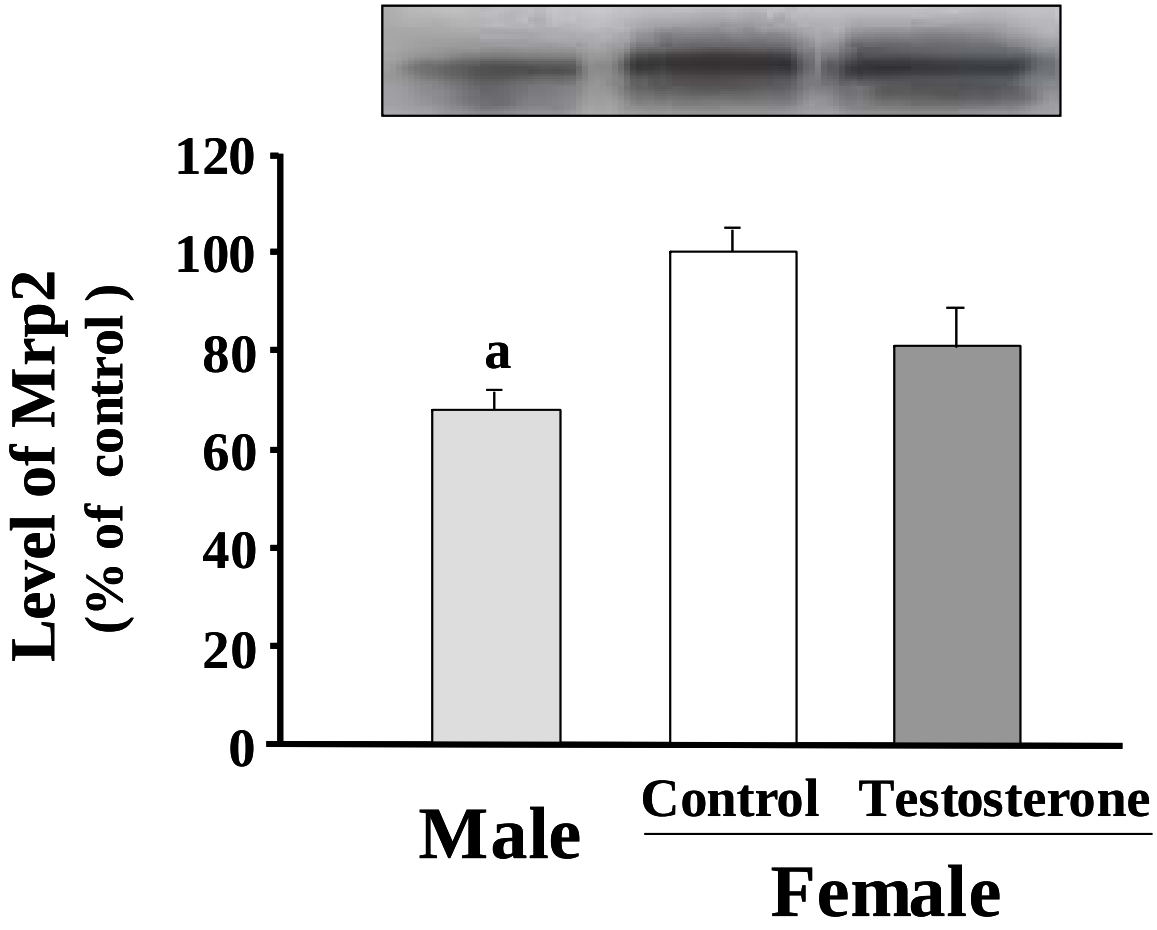
Level of Mrp2
(% of control)
120
100
80
60
40
20
0
a
Male
Control
Testosterone
Female

Fig. 4.