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Title:

Involvement of Breast Cancer Resistance Protein (*ABCG2*) in the biliary excretion mechanism of fluoroquinolones

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Running title:

biliary excretion of fluoroquinolones by BCRP

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Non-standard abbreviations:

ABC, ATP-binding cassette; BCRP (Bcrp), breast cancer resistance protein; BSP, sulfobromophthalein; CPFX, ciprofloxacin; EDTA, ethylenediaminetetracetic acid; EHBR, Eisai hyperbilirubinemic rat; GFP, green fluorescent protein; GPFX, Grepafloxacin; HEK293, human embryonic kidney 293; HEPES, 2-[4-(2-Hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; MDCK, Madin-Darbey Canine Kidney; MOI, multiplicity of infection; MRP (Mrp), multidrug resistance associated protein; OFX, ofloxacin; PhIP, 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine; Tris, 2-Amino-2-(hydroxymethyl)-1,3-propanediol; UFX, ulifloxacin;

Abstract

Fluoroquinolones are one of the effective antibiotics for the treatment of bile duct infections. It has been shown that the biliary excretion of grepafloxacin is partly accounted for by multidrug resistance associated protein 2 (MRP2/*ABCC2*), whereas neither MRP2 nor P-glycoprotein is involved in the biliary excretion of ulifloxacin. In the present study, we examined the involvement of breast cancer resistance protein (BCRP/*ABCG2*) in the biliary excretion of fluoroquinolones (grepafloxaxcin, ulifloxacin, ciprofloxacin and ofloxacin). In MDCK II expressing human BCRP or mouse Bcrp, the basal-to-apical transport of grepafloxacin and ulifloxacin was greater than that of the mock control, which was inhibited by a BCRP inhibitor, Ko143. Plasma and bile concentrations of fluoroquinolones were determined in wild type and Bcrp (-/-) mice following intravenous bolus injection. The cumulative biliary excretion of fluoroquinolones was significantly reduced in Bcrp (-/-) mice, resulting in a reduction of the biliary excretion clearances to 50, 16, 40 and 36% of the control values, for ciprofloxacin, grepafloxacin, ofloxacin and ulifloxacin, respectively. Preinfusion of sulfobromophthalein significantly inhibited the biliary excretion of grepafloxacin in Bcrp (-/-) mice. There was no change in the tissue-to-plasma concentration ratios of fluoroquinolones in the liver or brain, while those in the kidney were increased 3.6- and 1.5-fold for ciprofloxacin and grepafloxacin, respectively, in Bcrp (-/-) mice, but were unchanged for ofloxacin and ulifloxacin. The present study shows that BCRP mediates the biliary excretion of fluoroquinolones, and suggests that it is also involved in the tubular secretion of ciprofloxacin and grepafloxacin.

Introduction

Fluoroquinolones, inhibitors of bacterial DNA gyrase, exhibit strong antibacterial activities against variety bacteria, especially aerobic Gram-negative organisms. Orally administrated fluoroquinolones are widely distributed throughout the body following rapid absorption from the intestine. Many fluoroquinolones undergo either hepatic or renal elimination. Metabolism accounts for the hepatic elimination of fluoroquinolones, and biliary excretion is usually a minor elimination pathway. However, it has been reported that the concentrations of ciprofloxacin in the bile are far greater than the usual minimal inhibitory concentration of common biliary pathogens in humans, presumably due to active transport since the ratio of the concentrations of ciprofloxacin in bile/liver was greater than unity (Dan et al., 1987). Prulifloxacin, an ester type prodrug of ulifloxacin, is immediately metabolized to ulifloxacin in the intestine, and ten percent is recovered in the bile (Nakashima et al., 1994). Grepafloxacin and sparfloxacin are mainly eliminated from the liver by metabolism and biliary excretion, but very little is excreted into urine (Efthymiopoulos et al., 1997; Kamberi et al., 1998).

Although their ability to undergo biliary excretion is one of the crucial factors for their therapeutic efficacy against bile duct infections, the underlying mechanism of fluoroquinolones has not been fully investigated. A number of reports have shown that several kinds of transporters are expressed on the canalicular membrane in the liver and are responsible for the efficient elimination of drugs into the bile (Chandra and Brouwer, 2004; Pauli-Magnus and Meier, 2006; Shitara et al., 2006). It is

generally accepted that the transport of a variety of anionic drugs across the canalicular membrane is mainly mediated by multidrug resistance-associated protein 2 (MRP2/*ABCC2*), and some hydrophobic cationic drugs are eliminated into the bile by P-glycoprotein (Schinkel et al., 1995; Chandra and Brouwer, 2004). The biliary excretion of grepafloxacin is partly mediated by MRP2 since the biliary excretion of grepafloxacin was significantly reduced in MRP2 deficient mutant rats (EHBR) (29% of normal rats) (Sasabe et al., 1998). Although grepafloxacin is a substrate of P-gp, its biliary excretion is unchanged in Mdr1a/1b (-/-) mice, excluding the involvement of P-gp (Sasabe et al., 2004). For ulifloxacin, neither MRP2 nor P-gp is involved in its biliary excretion (Yagi et al., 2003). Therefore, other transporters have been hypothesized to account for the biliary excretion of UFX and GPFX.

Breast cancer resistance protein (BCRP/*ABCG2*) is the half-size ABC transporter identified on the bile canalicular membrane (Maliepaard et al., 2001; Jonker et al., 2002). *In vivo* studies using Bcrp (-/-) mice have shown that BCRP accounts for the biliary excretion of PhIP, pitavastatin and methotrexate (Hirano et al., 2005; Mao and Unadkat, 2005). Merino et al demonstrated that fluoroquinolones, such as ciprofloxacin (CPFX), ofloxacin (OFX), enrofloxacin and norfloxacin, are BCRP substrates, and that BCRP is involved in the secretion of fluoroquinolones into milk (Merino et al., 2006; Pulido et al., 2006). Therefore, it is possible that BCRP plays a significant role in the biliary excretion of fluoroquinolones. In the present study, *in vivo* studies using Bcrp (-/-) mice were

carried out to determine the contribution of BCRP to the biliary excretion of fluoroquinolones. In addition to the liver, BCRP is expressed in the luminal membrane of the brain capillaries and renal proximal tubules (Jonker et al., 2002; Lee et al., 2005). The brain is a target organ for the adverse effects (epileptogenic neurotoxicity), of fluoroquinolones and the kidney is another elimination organ. The tissue-to-plasma partition coefficients were determined in these tissues to determine the involvement of BCRP in the efflux transport of fluoroquinolones.

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Material and Methods

Animals

Male Bcrp (-/-) and wild-type FVB mice (16-18 weeks old) were used in the present study. Bcrp (-/-) mice were established previously (Jonker et al., 2002). All animals were maintained under standard conditions with a reverse dark light cycle and were treated humanely. Food and water were available ad libitum. The studies reported in this manuscript were carried out in accordance with the guidelines provided by the Institutional Animal Care Committee (Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan).

Materials

UFX was a gift from Meiji Seika (Tokyo, Japan). GPFX and OFX were gifted from Otsuka Pharmaceutical Company (Tokyo, Japan). CPFX and sulfobromophthalein were purchased from ICN Biomedicals, Inc (Aurora, OH) and Sigma-Aldrich (St.Louis, MO), respectively. Ko143 was supplied from Dr.Schinkel (Allen et al., 2002). Parent MDCK ll cells were kindly provided by Dr.Piet Borst. All other chemicals were of analytical grade and were commercially available.

Cell Culture

MDCK II cells (Matsushima et al., 2005) were grown in Dulbecco's modified Eagle's medium low glucose (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Sigma-Aldrich), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C with 5% CO_2 and at 95% humidity.

Transcellular Transport Study

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MDCK II cells were seeded in 24-well plates at a density of 1.4 x 10^5 cells/well (Matsushima et al., 2005). For constructing MDCK ll cells expressing human and mouse BCRP, and GFP as a negative control (hBCRP-MDCK, mBcrp-MDCK, GFP-MDCK, respectively), MDCK ll cells were infected with recombinant adenovirus containing human and mouse BCRP and GFP cDNA (200 MOI), 48 hr prior to all experiments (Matsushima et al., 2005). Krebs-Henseleit buffer consisted of 142 mM NaCl, 23.8 mM Na_2CO_3, 4.83 mM KCl, 0.96 mM KH_2PO_4, 1.20 mM $MgSO_4$, 12.5 mM HEPES, 5 mM glucose, and 1.53 mM $CaCl_2$ adjusted to pH 7.4. The experiments were initiated by replacing the medium on either the apical or basal side of the cell layer with complete medium containing fluoroquinolone (10 µM) with or without Ko143 (1 µM). The cells were incubated at 37°C, and aliquots of medium were taken from each compartment at designated time points. The medium (100µL) was mixed with 50µL methanol containing 0.15% formic acid, followed by centrifugation at 4°C and 10,000*g* for 10min. The supernatant was subjected to analysis by LC/MS. At the end of the experiments, the cells were solubilized in 450 µl 0.2 N NaOH. After the addition of 225 µl 0.4 N HCl, aliquots of cell lysate were used to determine protein concentrations. Protein concentrations were determined by the Lowry method, and bovine serum albumin was used as a standard..

In Vivo Study in Mice

Male FVB and Bcrp (-/-) mice weighing approximately 25 to 36 g were used throughout these experiments. Under urethane anesthesia (1.5 mg/kg), the

gallbladder was ligated and the bile duct was cannulated with a Teflon catheter (UT-03, Unique medical co., Ltd, Tokyo, Japan) for bile collection. The mice received a bolus administration of each fluoroquinolone at a dose of 1 mg/kg via the jugular vein. Blood samples were collected from the opposite jugular vein at 7.5, 15, 30, 60 and 120 min. Bile was collected in preweighed test tubes at 30-min intervals throughout the experiments. Plasma was prepared by centrifugation of the blood samples. The mice were sacrificed after 120 min, and the entire liver, kidney and brain were excised immediately. The tissues were weighed and stored at -80°C until quantification. Portions of liver, kidney and brain were added to two volumes of PBS (w/v) and homogenized. Plasma (25 µl), bile (10 µl), or tissue homogenate (100 µl) was deproteinized with two volumes of methanol containing 0.15% formic acid (w/v), followed by centrifugation at 4°C and 10,000*g* for 10 min. The supernatant was diluted with 0.05% formic acid solution and analyzed using the LC/MS system (Shimadzu Scientific Instruments). No chromatographic interference was found with regard to these fluoroquinolones in extracts from blank plasma, bile, and tissue homogenates. We measured the calibration curve before and after each sample analysis, and did not observe any significant inter- and intraday difference. The calibration range was from 0.01 to 3µM. The dilution factor range of supernatants of plasma, bile and tissues in this study was 1-10, 15-30 and 2-3, respectively. Each calibration curve was constructed using proper matrices, such as plasma supernatant and diluted plasma supernatant.

Inhibition study by BSP of biliary excretion of GPFX

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BSP dissolved in 0.9% saline was infused into a jugular vein through a catheter at a rate of 90.5 mg/hr/kg (5 ml/hr/kg) throughout the experiments (Horikawa et al., 2002). Twenty minutes after starting the infusion, GPFX was administered at a dose of 1 mg/kg via the jugular vein. The sample preparation and quantification of GPFX was performed as described above.

Pharmacokinetic Analysis

The total plasma clearance (CL_{total}), biliary excretion clearance normalized by circulating plasma ($CL_{bile, plasma}$), and apparent intrinsic biliary excretion clearance ($CL_{bile, liver, app}$) were calculated using following equations:

$$CL_{total} = Dose/AUC_{0-\infty}$$

$$CL_{bile,plasma} = X_{bile}/AUC_{0-120}$$

$$CL_{bile,liver,app} = X_{bile}/AUC_{0-120}/Kp_{liver,app}$$

where Dose, $AUC_{0-\infty}$, AUC_{0-120}, X_{bile}, and $Kp_{liver, app}$ represent the dosage (in milligrams per kilogram), the area under the plasma concentration-time curve from 0 to ∞ min and 0 to 120 min(in micromolar units multiplied by the time in minutes), the amount excreted into the bile (in nanomoles per kilogram), and the liver-to-plasma concentration ratio at 120 min (in grams per milliliter), respectively. AUC was calculated using trapezoidal method. Plasma concentration at 0 min and the time when plasma concentration was diminished were acquired by the extrapolation.

Determination of fluoroquinolones in the specimens

The LC/MS system consisted of LC-20AD dual micro-plunger pumps, a SIL-20AC auto sampler, CTO-20AC column oven and LCMS-2010EV mass

spectrometer (Shimadzu Corporation, Kyoto, Japan). The LC/MS analysis of fluoroquinolones was performed using an MGII C^{18} column (50 x 2.1 mm; particle size, 3 μm) at the flow rate of 0.5 ml/min (Shiseido Co., Ltd, Tokyo, Japan). Two mobile phases were used for gradient elution as follows; for CPFX and OFX, 0.05% formic acid/acetonitrile (%/%) 92.5/7.5 at 0-0.5 min, 80/20 at 0.5-2.5 min, 92.5/7.5 at 2.5-6.0 min. For GPFX and UFX, 0.05% formic acid/acetonitrile (%/%) 88.5/11.5 at 0-0.5 min, 80/20 at 0.5-2.5 min, and 88.5/11.5 from 2.5 min. The MS instrument used for this work was an LCMS-2010EV mass spectrometer (Shimadzu), which was operated in the positive-ion electro spray ionization mode. The m/z monitored was 332, 360, 362 and 360 for CPFX, GPFX, OFX and UFX, respectively. The retention times of CPFX, GPFX, OFX and UFX were 2.5, 2.9, 2,2 and 2.7 min, respectively. The detection limit for these fluoroquinolones was 0.01 μM in the transport buffer and plasma, and 0.03μM in bile and tissue homogenates.

Statistical Analysis

Statistically significant differences were determined using two-tailed unpaired t-tests in this study. Differences were considered to be significant at $P < 0.05$.

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Results

Transcellular transport of fluoroquinolones across monolayers of MDCK II cells expressing human and mouse BCRP.

The transcellular transport of GPFX and UFX across the monolayers of hBCRP-, mBcrp- and GFP-MDCK was determined. Expression of human and mouse BCRP induced asymmetric transepithelial transport of GPFX, enhanced the basal-to-apical transport, and reduced the apical-to-basal transport (Figure. 1). We compared the translocation of GPFX in the basal-to-apical direction for 120 min was approximately 10- and 4.0-fold greater than that in the opposite direction in hBCRP- and mBcrp-MDCK, respectively, which was identical in GFP-MDCK (Figure. 1). The translocation of UFX in the basal-to-apical direction for 120 min was slightly increased; 1.8- and 1.5-fold of that in the opposite direction in hBCRP- and mBcrp-MDCK cells, respectively (Figure. 2), whereas that in the control was 0.6-fold. The ratio of the transcellular transport at 180 min (basal-to-apical / apical-to-basal) was greater than that in the presence of Ko143: 9.1- and 3.8-fold for GPFX, and 3.0- and 2.4-fold for UFX respectively, 9.87 versus 1.17, 4.05 versus 1.09, 1.84 versus 0.84 and 1.46 versus 0.47 (Table. 1). Ko143 (1 μM), an inhibitor of BCRP, reduced the directional transport of the fluoroquinolones observed in MDCK II cells expressing hBCRP and mBcrp.

Plasma concentrations and biliary excretion time profiles, and liver concentration in mice after bolus administration of fluoroquinolones.

Male Bcrp (-/-) and FVB mice received a bolus administration of each

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fluoroquinolone antibiotics at a dose of 1 mg/kg via the jugular vein. Blood and bile were collected at designated time points. Plasma concentrations time profiles of all fluoroquinolones were similar in Bcrp (-/-) and FVB mice, but the cumulative biliary excretion of all fluoroquinolones was significantly reduced in Bcrp (-/-) mice (Figure. 3). The pharmacokinetic parameters are summarized in Table 2. There was no significant difference in the plasma clearance of all fluoroquinolones in Bcrp (-/-) and FVB mice. The $CL_{bile,\ p}$ of CPFX, GPFX and UFX was significantly reduced, but the reduction in the $CL_{bile,\ p}$ of OFX was not statistically significant.

The tissue-to-plasma concentration ratios in BCRP expressing tissues such as liver, brain and kidney were determined. In the liver and brain, the tissue-to-plasma concentration ratios of all fluoroquinolones were unchanged, whereas those of CPFX and GPFX in kidney were increased in Bcrp (-/-) mice (Table 2).

Inhibition study of MRP2 mediated biliary excretion of GPFX in Bcrp (-/-) mice using BSP infusion.

BSP administration caused no significant differences in the plasma total body clearance in Bcrp (-/-) mice with and without BSP infusion, but the cumulative biliary excretion of GPFX was reduced by BSP (Figure. 4). The $CL_{bile,\ p}$ of GPFX was slightly changed but this was not statistically significant. The liver-to-plasma concentration ratio of GPFX was higher in the BSP-treated group, while the $CL_{bile,\ liver,\ app}$ was significantly reduced in the BSP-treated group (Table 3).

Discussion

In this study, we investigated the involvement of BCRP in the biliary excretion and tissue distribution of fluoroquinolones using Bcrp (-/-) mice.

To show that they are substrates of BCRP, their transcellular transport across monolayers of MDCK ll cells was determined. BCRP is expressed on the apical membrane of MDCK ll cells (Matsushima et al., 2005), and Merino et al reported that the basal-to-apical transport of CPFX and OFX was increased in hBCRP- and mBcrp-MDCK, which was reduced in the presence of Ko143, a BCRP specific inhibitor (Merino et al., 2006). Consistent with other fluoroquinolones, exogenous expression of hBCRP/mBcrp induced directional transport of GPFX in the basal-to-apical direction, which was reduced by a BCRP inhibitor, Ko143 (Figure 1). Expression of hBCRP/mBcrp also significantly increased the Ko143-sensitive basal-to-apical transport of UFX (Figure 2). The ratios of the transcellular transport of UFX in hBCRP/mBcrp expressing cells were smaller than those of GPFX (Table 1), indicating that GPFX is a better hBCRP/mBcrp substrate with greater transport activities. Unlike GPFX, the apical-to-basal transport of UFX was greater than its basal-to-apical transport in control MDCK cells, suggesting that endogenous transporter(s) facilitate the apical-to-basal transport (Table 1).

The biliary excretion clearance of fluoroquinolones was compared between wild-type and Bcrp (-/-) mice. The cumulative biliary excretion of the fluoroquinolones, particularly UFX, was significantly reduced in Bcrp (-/-) mice, suggesting that BCRP plays a major role in the biliary excretion of UFX, and partly

accounts for the biliary excretion of other fluoroquinolones. The plasma concentration-time profiles of the fluoroquinolones were not affected by knockout of Bcrp. This is reasonable considering that the biliary excretion clearance of fluoroquinolones only accounts for a small part of their total body clearance. The minimal change in the liver concentration of the fluoroquinolones suggests only a small contribution to the total sequestration pathways from the liver. Indeed, Sasabe et al suggested that sinusoidal efflux is the major elimination pathway from the liver in rats in a study that compared the hepatic clearance of GPFX with the hepatic uptake clearance (Sasabe et al., 1997).

Although GPFX is a mBcrp substrate with greater transport activity than UFX, the magnitude of the reduction in the biliary excretion clearance of GFPX was lower than that of UFX. Since involvement of MRP2 in the biliary excretion of GPFX has been suggested by *in vivo* kinetic analysis using EHBR, the discrepancy may be explained by the smaller contribution of mBcrp to the net canalicular efflux process of GPFX. This possibility was investigated by examining an inhibitory effect of BSP on the biliary excretion of GPFX. BSP caused only a minimal difference in the $CL_{bile,\ p}$ in Bcrp (-/-) mice. However, taking the greater hepatic concentration in the BSP-treated group into the consideration, the biliary excretion clearance with regard to the hepatic concentration will be significantly inhibited, suggesting an involvement of BSP-sensitive efflux transporter at the canalicular membrane, presumably MRP2. The increased hepatic concentration produced by BSP may be ascribed to an inhibition of hepatic metabolism and/or sinusoidal efflux of GPFX.

Considering the kinetic analyses by Sasabe et al (1997), it can be speculated that BSP inhibits the sinusoidal efflux of GPFX. This hypothesis should be investigated using the in situ perfusion technique to compare the concentration time profile in the outflow in mice treated with and without BSP.

In addition to the liver, we observed that the kidney-to-plasma concentration ratio of CPFX and GPFX is 3.6 and 1.5 times higher in Bcrp (-/-) mice than that in FVB mice. Although very little GPFX is excreted into the urine in rats (Sasabe et al., 1998), the urinary excretion accounts for the 50% of the total body clearance in EHBRs (Nouaille-Degorce et al., 1998). BCRP may play a significant role in the urinary excretion of CPFX as well as its biliary excretion. It should be noted that the protein expression of BCRP was below the limit of detection in human kidney (Maliepaard et al., 2001). Since CPFX undergoes tubular secretion in humans, which is inhibited by probenecid (Jaehde et al., 1995), other transporters will account for the luminal efflux of CPFX in humans.

Epileptogenic neurotoxicity is a well-known central nervous system side effect of fluoroquinolones and, thus, the transport of BBB is a critical factor for this adverse effect together with the inhibition potency of GABA receptors. Fluoroquinolones have been suggested to undergo active efflux at the BBB because the steady-state brain interstitial space concentration of fluoroquinolones was markedly lower than their plasma unbound concentrations (Ooie et al., 1997b), and the permeability surface area product of the efflux transport was 10-300 fold greater than the uptake clearance (Ooie et al., 1997a). Using P-gp knockout mice, P-gp has been shown

to limit the brain uptake of GPFX and UFX (Murata et al., 1999; Sasabe et al., 2004). The present study has demonstrated the limited role of Bcrp in the efflux transport of fluoroquinolones at the BBB, and, unlike the liver, P-gp is likely responsible for major efflux transport of fluoroquinolones at the BBB. The mRNA quantification of Mdr1a showed more abundant expression in the cerebral cortex than in the liver (Brady et al., 2002), and vice versa for Bcrp (Tanaka et al., 2005). This may explain the tissue-selective contribution of P-gp and Bcrp to the net efflux process in the liver and brain.

This study showed that Bcrp is involved in the biliary excretion of CPFX, GPFX, OFX and UFX. The hepatic expression of BCRP is influenced by gender, the greater expression in males than females (Merino et al., 2005). Consistent with hepatic expression, the biliary excretion of nitrofurantoin and PhIP was greater in male mice (Merino et al., 2005). Therefore, gender could be a critical factor affecting the therapeutic efficacy of fluoroquinolones for the treatment of bile duct infections. In addition to gender, there are genetic factors affecting the therapeutic efficacy. A genetic polymorphism (C421A) substituting Gln for Lys is associated with the low protein expression of BCRP, and this SNP is frequently found particularly in the Japanese population (20-30%) (Kondo et al., 2004). Further studies are necessary to investigate interindividual difference of BCRP activity in the liver.

In conclusion, Bcrp plays a significant role in the biliary excretion of fluoroquinolones, and in the urinary excretion of CPFX and GPFX, but only a limited

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role in the efflux transport at the BBB.

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Footnotes

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Legends for figures

Figure.1 Time profiles of transcellular transport of GPFX across MDCK II monolayers expressing transporters.

Transcellular transport of GPFX (10μM) with or without Ko143 (1μM) across MDCK II monolayers expressing hBCRP (A), (B) and mBcrp (C), (D) was compared with that across control MDCK II monolayers expressing GFP (E), (F). Open and closed circles represent the transcellular transport with or without Ko143 (1μM), respectively. Graph (A), (C) and (E) represent the transcellular transport in the apical-to-basal direction, and graph (B), (D), (F) represent that in the basal-to-apical direction. Each point plotted, with its vertical bar, represents the mean±S.E.(n=6). **P*<0.05, ***P*<0.01 versus control values.

Figure.2 Time profiles of transcellular transport of UFX across MDCK II monolayers expressing transporters.

Transcellular transport of UFX (10μM) with or without Ko143 (1μM) across MDCK II monolayers expressing hBCRP (A), (B) and mBcrp (C), (D) was compared with that across control MDCK II monolayers expressing GFP (E), (F). Open and closed circles represent the transcellular transport with or without Ko143 (1μM), respectively. Graph (A), (C) and (E) represent the transcellular transport in the apical-to-basal direction, and graph (B), (D), (F) represent that in the basal-to-apical direction. Each point plotted, with its vertical bar, represents the mean±S.E.(n=4). **P*<0.05, ***P*<0.01 versus control values.

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Figure.3 Time profiles of the plasma concentration and cumulative biliary excretion of CPFX, GPFX, OFX and UFX after a single intravenous dose in Bcrp (-/-) and wild-type mice.

CPFX, GPFX, OFX and UFX were given to Bcrp (-/-) (open symbol) and wild-type mice (closed symbol) at a dose of 1 mg/kg. The plasma concentration (A) and cumulative biliary excretion (B) of CPFX, GPFX (C) (D), OFX (E) (F) and UFX (G) (H) were determined by LC/MS. Each point plotted, with its vertical bar, represents the mean$\pm$S.E. of four mice. **P*<0.05, ***P*<0.01 versus control values.

Figure.4 Inhibition of biliary excretion of GPFX by BSP, in male Bcrp (-/-) mice.

BSP (1.51mg/min/kg) was infused intravenously 20min prior to the i.v. bolus injection of GPFX (1mg/kg). The plasma concentration in Bcrp (-/-) mice (A) and cumulative biliary excretion (B) of GPFX after bolus administration with (open symbols) or without pre-infusion of BSP (closed symbols) were determined by LC/MS. Each point plotted, with its vertical bar, represents the mean$\pm$S.E. of four mice. **P*<0.05, ***P*<0.01 versus control values.

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Tables

Table 1.Transcellular transport of fluoroquinolones across MDCK II monolayers expressing transporters and inhibition of transcellular transport by Ko143

		apical to basal [a)] (μl/mg protein)	basal to apical [b)] (μl/mg protein)	Ratio c) (b to a/a to b)
GPFX	**GFP**	**84.3 ± 14.6**	**91.4 ± 7.5**	**1.08 ± 0.21**
	hBCRP	**25.4 ± 3.3**	**251 ± 38**	**9.87 ± 1.98** ††**
	mBcrp	**43.8 ± 6.5**	**178 ± 25**	**4.05 ± 0.82** †**
GPFX + Ko143	**GFP**	**102 ± 3**	**113 ± 2**	**1.10 ± 0.04**
	hBCRP	**115 ± 8**	**135 ± 12**	**1.17 ± 0.13**
	mBcrp	**108 ± 5**	**118 ± 5**	**1.09 ± 0.06**
UFX	**GFP**	**76.9 ± 15.6**	**47.8 ± 5.1**	**0.62 ± 0.14**
	hBCRP	**90.7 ± 3.5**	**166 ± 25**	**1.84 ± 0.28****
	mBcrp	**53.4 ± 7.4**	**78.3 ± 11.5**	**1.46 ± 0.30* †**
UFX + Ko143	**GFP**	**101 ± 15**	**70.0 ± 18.4**	**0.69 ± 0.21**
	hBCRP	**114 ± 24**	**95.1 ± 30.2**	**0.84 ± 0.32**
	mBcrp	**83.0 ± 11.2**	**38.6 ± 3.8**	**0.47 ± 0.08**

Data are taken from Figures 1 and 2. Each value represents the mean±S.E.

a): transcellular transport in the apical-to-basal direction at 180min

b): transcellular transport in the basal-to-apical direction at 180min

*: $P<0.05$ **: $P<0.01$(significantly different from the corresponding parameters in GFP expressing cells)

†: $P<0.05$ ††: $P<0.01$(significantly different from the corresponding parameters with Ko143 (1μM))

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Table 2. Pharmacokinetic parameters of CPFX, GPFX, OFX and UFX after a single intravenous administration at a dose of 1mg/kg

		CL_{total} [a] (ml/min/kg)	$CL_{bile, p}$ [b] (ml/min/kg)	$K_{p, liver}$ [c]	$K_{p, kidney}$ [d]	$K_{p, brain}$ [e]
CPFX	**Wild-type**	**22.0 ± 1.2**	**3.65 ± 0.83**	**5.32 ± 0.14**	**5.79 ± 0.57**	**0.14 ± 0.01**
	Bcrp (-/-)	**31.2 ± 4.3**	**1.30 ± 0.11***	**6.19 ± 1.41**	**20.8 ± 2.1****	**0.32 ± 0.15**
GPFX	**Wild-type**	**6.82 ± 0.02**	**0.61 ± 0.04**	**1.99 ± 0.18**	**3.19 ± 0.33**	**0.11 ± 0.01**
	Bcrp (-/-)	**7.63 ± 0.01**	**0.30 ± 0.02****	**2.10 ± 0.22**	**4.72 ± 0.20****	**0.13 ± 0.01**
OFX	**Wild-type**	**28.1 C 6.8**	**1.98 ± 0.51**	**3.03 ± 0.79**	**10.9 ± 6.4**	**0.26 ± 0.07**
	Bcrp (-/-)	**18.8 ± 1.7**	**0.76 ± 0.04**	**1.45 ± 0.15**	**8.02 ± 1.77**	**0.15 ± 0.02**
UFX	**Wild-type**	**16.6 ± 4.6**	**3.07 ± 0.60**	**2.86 ± 0.56**	**15.7 ± 6.1**	**n.d.**
	Bcrp (-/-)	**21.2 ± 8.1**	**0.50 ± 0.09****	**4.21 ± 1.84**	**15.1 ± 7.2**	**n.d.**

Data are taken from Figure 3. Each value represents the mean±S.E. of four mice.

a): total body clearance obtained by dividing the dose by the AUC(0-∞)

b): biliary clearance obtained by dividing the cumulative biliary excretion by the AUC (0-120min)

c): plasma-to-liver concentration ratio at 120min.

d): plasma-to-kidney concentration ratio at 120min

e): plasma-to-brain concentration ratio at 120min

n.d. : not detected

*P<0.05, **P<0.01 versus control values.

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Table 3. Effect of BSP on the pharmacokinetic parameters of GPFX in Bcrp- (-/-) mice.

	CL_{total} a) (ml/min/kg)	$CL_{bile, p}$ b) (ml/min/kg)	$K_{p, liver}$ c)	$CL_{bile, liver, app}$ d)
+Saline	**11.5 ± 2.7**	**0.19 ± 0.03**	**3.90 ± 0.38**	**0.049 ± 0.009**
+BSP	**16.4 ± 3.3**	**0.15 ± 0.01**	**6.29 ± 0.67***	**0.024 ± 0.003***

BSP was given to Bcrp (-/-) mice by intravenous infusion at an infusion rate of1.51mg/min/kg 20 min prior to the bolus administration of GPFX. Data are taken from Figure 4.

Each value represents the mean±S.E. of four mice.

a): total body clearance obtained by dividing the dose by the AUC(0-∞)

b): biliary clearance obtained by dividing the cumulative biliary excretion by the AUC(0-120min)

c): plasma-to-liver concentration ratio at 120min.

d): biliary clearance obtained by dividing the cumulative biliary excretion by the liver concentration at 120min.

*P<0.05, **P<0.01 versus control values.

Figure.1

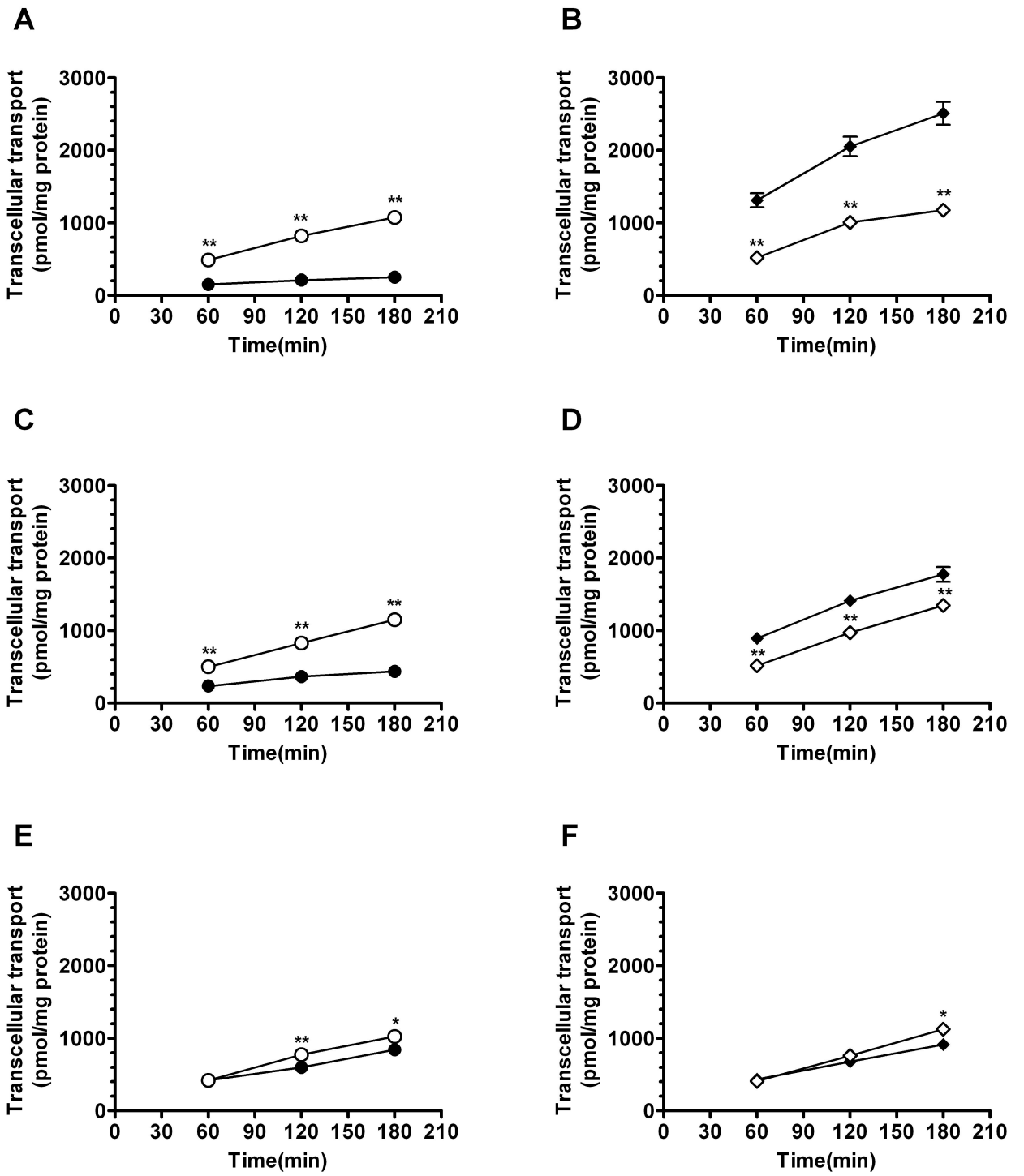
A
B
C
D
E
F
Transcellular transport (pmol/mg protein)
Time(min)
0
30
60
90
120
150
180
210
1000
2000
3000
**
*

Figure.2

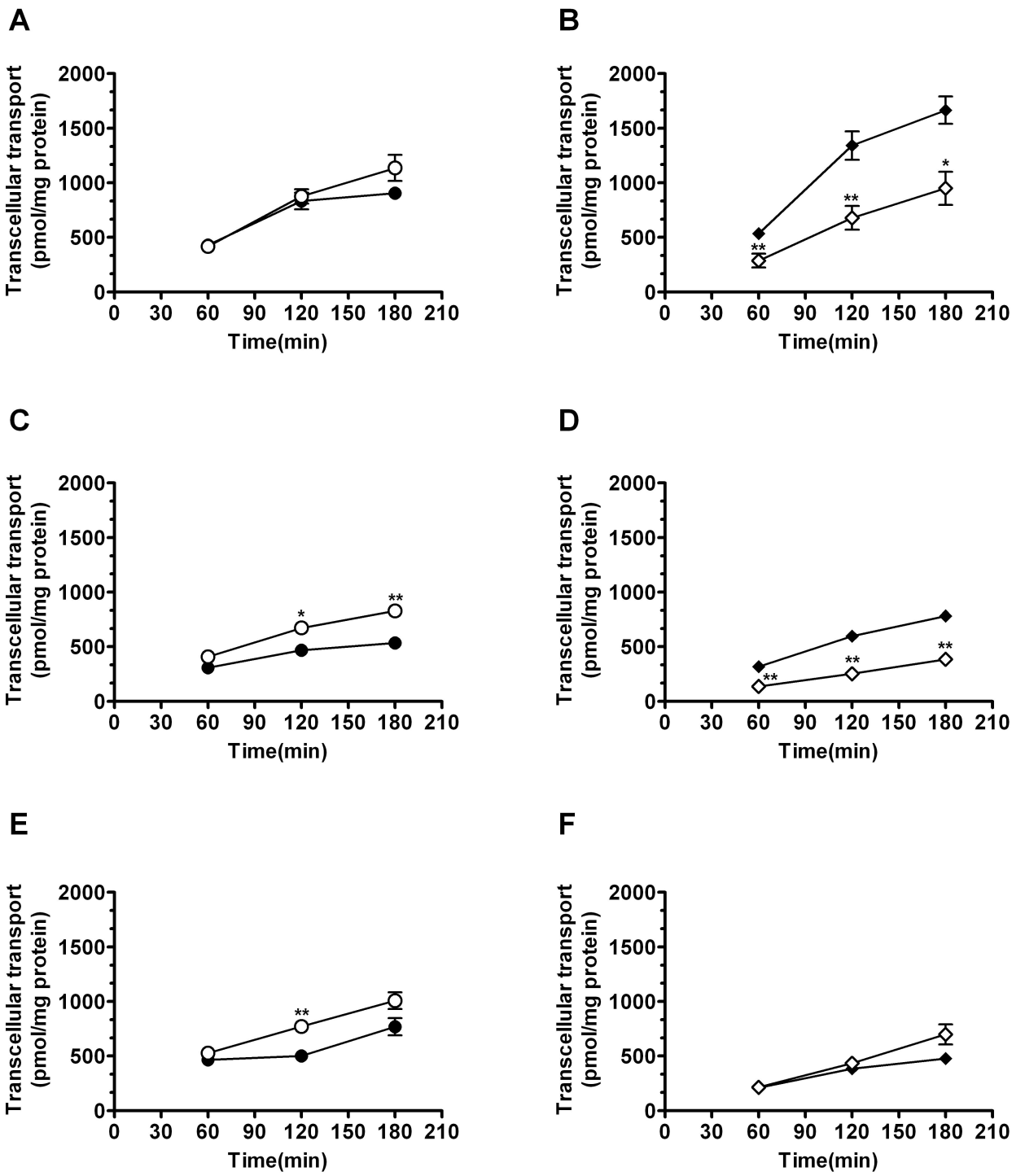
A
B
C
D
E
F
Transcellular transport
(pmol/mg protein)
2000
1500
1000
500
0
0 30 60 90 120 150 180 210
Time(min)
*
**

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Figure.3

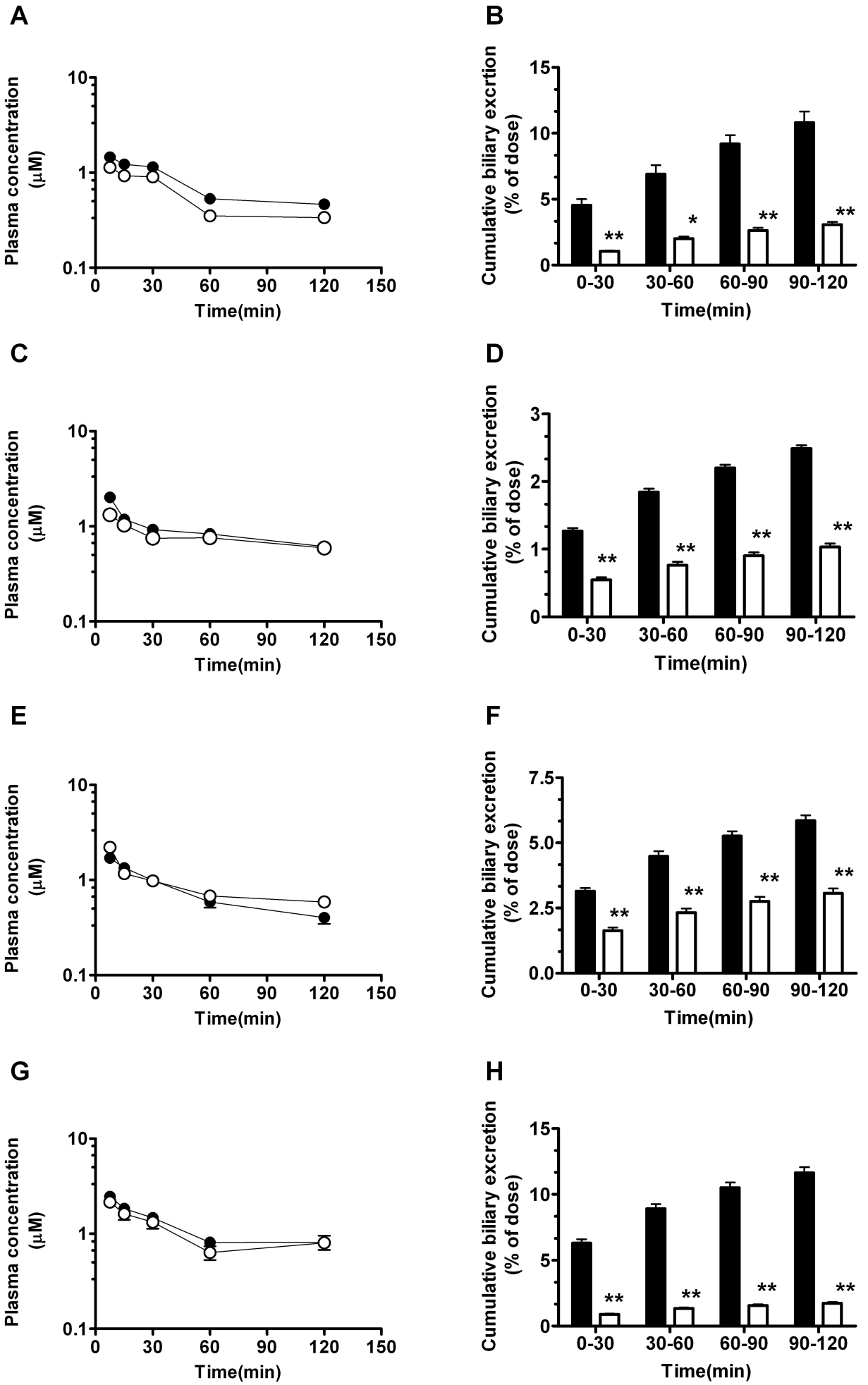
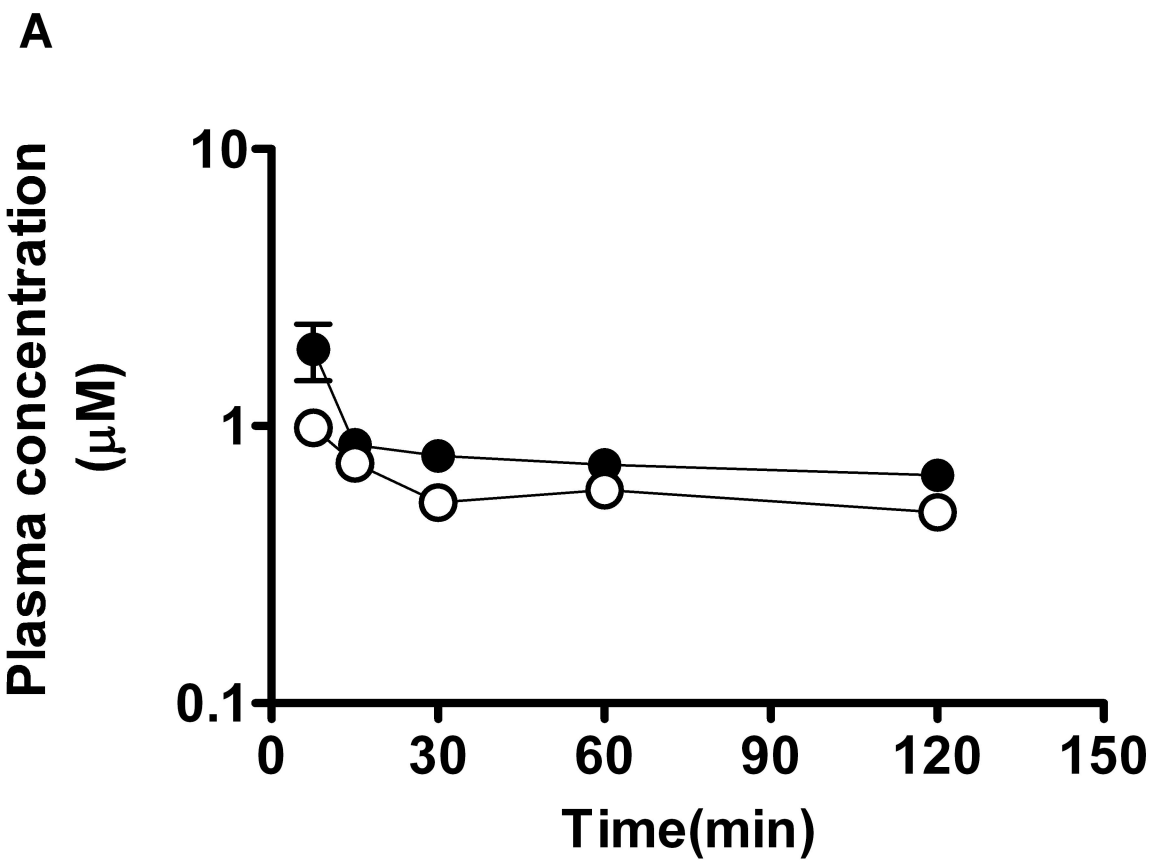
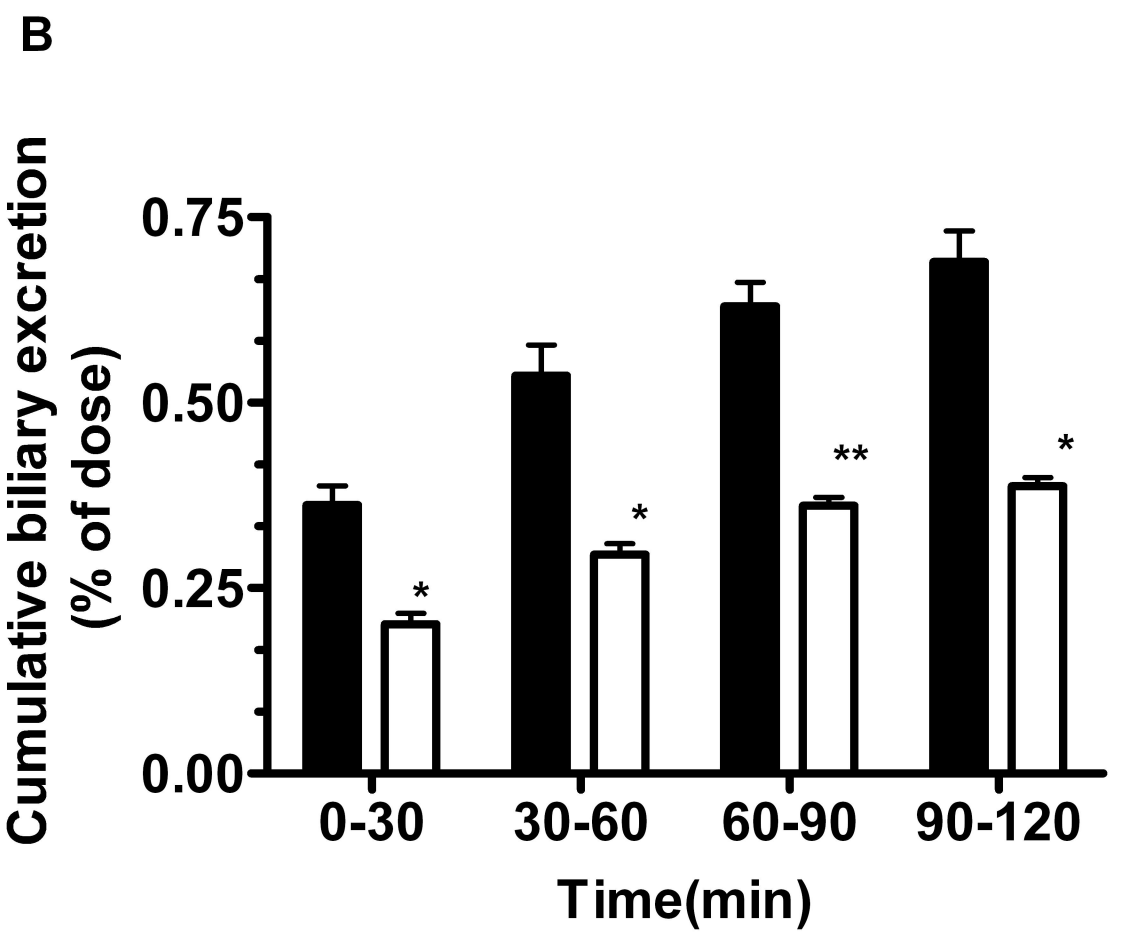


Figure.4


A
Plasma concentration (μM)
10
1
0.1
0
30
60
90
120
150
Time(min)


B
Cumulative biliary excretion (% of dose)
0.75
0.50
0.25
0.00
*
*
**
*
0-30
30-60
60-90
90-120
Time(min)