Pharm Res (2015) 32:1634–1647
DOI 10.1007/s11095-014-1563-4

RESEARCH PAPER

Evaluation of the Usefulness of Breast Cancer Resistance Protein (BCRP) Knockout Mice and BCRP Inhibitor-Treated Monkeys to Estimate the Clinical Impact of BCRP Modulation on the Pharmacokinetics of BCRP Substrates

Tsuyoshi Karibe • Rie Hagihara-Nakagomi • Koji Abe • Tomoki Imaoka • Tsuyoshi Mikkaichi • Satoru Yasuda • Masakazu Hirouchi • Nobuaki Watanabe • Noriko Okudaira • Takashi Izumi

Received: 22 June 2014 / Accepted: 28 October 2014 / Published online: 8 November 2014

ABSTRACT

Purpose To evaluate whether the impact of functional modulation of the breast cancer resistance protein (BCRP, *ABCG2* 421C>A) on human pharmacokinetics after oral administration is predictable using Bcrp knockout mice and cynomolgus monkeys pretreated with a BCRP inhibitor, elacridar.

Methods The correlation of the changes of the area under the plasma concentration-time curve (AUC) caused by *ABCG2* 421C>A with those caused by the Bcrp knockout in mice, or BCRP inhibition in monkeys, was investigated using well-known BCRP substrates (rosuvastatin, pitavastatin, fluvastatin, and sulfasalazine).

Results In mice, the bioavailability changes, which corrected the effect of systemic clearance by Bcrp knockout, correlated well with the AUC changes in humans, whereas the correlation was weak when AUC changes were directly compared. In monkeys, the AUC changes pretreated with elacridar resulted in a good estimation of those in humans within approximately 2-fold ranges.

Conclusions This study suggests that pharmacokinetics studies that use the correction of the bioavailability changes in Bcrp knockout mice are effective for estimating clinical AUC changes in *ABCG2* 421C>A variants for BCRP substrate drugs and those studies in monkeys that use a BCRP inhibitor serve for the assessment of BCRP impact on the gastrointestinal absorption in a non-rodent model.

KEY WORDS *ABCG2* 421C>A · BCRP · Cynomolgus monkey · Gastrointestinal absorption · Knockout mouse

T. Karibe (✉) • R. Hagihara-Nakagomi • K. Abe • T. Imaoka • T. Mikkaichi • S. Yasuda • M. Hirouchi • N. Watanabe • N. Okudaira • T. Izumi
Drug Metabolism & Pharmacokinetics Research Laboratories, R&D Division, Daiichi Sankyo Co., Ltd., 1-2-58, Hiromachi Shinagawa-ku, Tokyo 140-8710, Japan
e-mail: karibe.tsuyoshi.jn@daiichisankyo.co.jp

ABBREVIATIONS

AUC	Area under the plasma concentration-time curve
AUC_{all}	AUC from 0 h to the time of the last observation
BCRP	Bcrp, breast cancer resistance protein
CL_{tot}	Total body clearance based on plasma concentration
C_{max}	The maximum plasma concentration
CYP	Cytochrome P450
EL	Elacridar pretreatment
F	Bioavailability
FLV	Fluvastatin
IC_{50}	Half maximal (50%) inhibitory concentration
KO	Knockout
LC/MS/MS	Liquid chromatography/tandem mass spectrometry
OATP	Organic anion transporting polypeptide
P-gp	P-glycoprotein
PK	Pharmacokinetics
PTV	Pitavastatin
RSV	Rosuvastatin
SASP	Sulfasalazine
Statin	HMG-CoA reductase inhibitor
$t_{1/2}$	The elimination terminal half-life
t_{max}	The time to reach C_{max}
UT	Untreatment
V_{dss}	Apparent volume of disposition at equilibrium
WT	Wild type

INTRODUCTION

The breast cancer resistance protein (BCRP, ABCG2) is constitutively expressed in healthy human tissues such as the brain, placenta, liver, intestine, breast, kidney, and stem cells, as well as multidrug-resistant cancer (1,2). It plays an

important role in absorption, distribution, and elimination of drugs (1,2). Known substrates of BCRP are nitrofurantoin (3) and sulfasalazine (SASP) (4), an antibiotic for urinary tract infection and a drug widely used for inflammatory bowel disease, respectively, and HMG-CoA reductase inhibitors such as rosuvastatin (RSV) (5), pitavastatin (PTV) (6), and fluvastatin (FLV) (7).

Recently, there are numerous reports declaring that the *ABCG2* 421C>A variant significantly affected the pharmacokinetics (PK) of SASP (8–10), RSV (11,12), and FLV (13) in clinical due to the decrease of BCRP mediated transport activity (14,15). The area under the plasma concentration-time curve (AUC) and maximum plasma concentration (C_{max}) of SASP, RSV, and FLV after the oral (p.o.) administration in *ABCG2* 421 AA or CA subjects were approximately 2–4 times higher compared to those of *ABCG2* 421 CC, whereas the time to reach C_{max} (t_{max}) and the elimination terminal half-life ($t_{1/2}$) remained unchanged. These data suggest that the *ABCG2* 421C>A genotype mainly affects the BCRP function during the absorption phase in humans. The allelic frequencies in African, Caucasian, Japanese, and Chinese people of *ABCG2* 421C>A are reported to be 0–4, 8–15, 25–36, and 35%, respectively (15,16), showing relatively high frequency in Asians leading to the possible wide interindividual variability in PK of BCRP substrates. Indeed, increased exposure to a BCRP substrate sunitinib, an oral multi-kinase inhibitor, in Asian patients was due to high frequency of *ABCG*2 421C>A and associated with a higher incidence of adverse events and poor compliance (17). However, not all of the BCRP substrates identified by various *in vitro* studies are accompanied with the PK changes in *ABCG2* variants in humans: Nitrofurantoin (18) and PTV (19) had minimal changes of exposure in *ABCG2* variants. Therefore, estimation of the impact of the functional modulation of BCRP on human PK is important in drug development. The U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have recommended the assessment of whether a drug candidate is a substrate and/or an inhibitor of BCRP, as well as P-glycoprotein (P-gp), as a possible cause of transporter related drug-drug interaction and interindividual variability in their guidance document (FDA Draft Guidance for Industry: Drug Interaction Studies, 2012) and guideline (EMA Guideline on the investigation of drug interactions, 2012).

In regard to estimating the contribution of BCRP, such studies have been carried out using Bcrp (−/−) mice (Bcrp knockout (KO) mice). Zaher *et al.* (20) reported that BCRP is a major determinant of the oral bioavailability as well as the elimination of SASP using Bcrp KO mice. Although the study clearly demonstrated the contribution of BCRP to PK of SASP, the degree of AUC increase in Bcrp KO mice was greatly overestimated compared to that observed clinically in *ABCG2* 421C>A subjects. This overestimation of AUC increase of SASP in Bcrp KO mice is presumably because the contribution of Bcrp to systemic clearance (i.e. hepatic clearance), not just to the absorption phase, affected the AUC increase. In view of the fact that the *ABCG2* 421C>A genotype mainly affects the BCRP function during the absorption phase in clinical, separate evaluation of the BCRP function in the absorption phase from that in the elimination phase is required. This study evaluated the Bcrp function in the absorption phase in mice and monkeys apart from contribution to the elimination phase.

In order to predict the impact of AUC increase in clinical, *in vivo* evaluation of the contribution of BCRP during the absorption phase using a non-rodent model is also important to drug development because large species differences between rodents and humans are often observed compared to those between monkeys and humans in the contribution of metabolism and/or the elimination pathway (21). In non-rodents, such attempts at evaluation of BCRP contribution *in vivo* are limited, although there are some reports that mention P-gp contribution in *in vivo* using monkeys and pretreatment with elacridar or ketoconazole as inhibitors of P-gp (22,23) or organic anion transporting polypeptide (OATP) contribution in *in vivo* using pretreatment with rifampicin (24,25). Still, comprehensive studies are lacking as to an elucidation of how PK alteration in nonclinical species, such as Bcrp KO mice and monkeys, is predictive of that in humans caused by BCRP modulation using typical BCRP substrates.

The purpose of the present study is to elucidate whether the impact of the functional modulation of BCRP on the absorption process of its substrates observed in *ABCG2* 421C>A subjects can be assessed by pre-clinical studies using Bcrp KO mice and whether cynomolgus monkeys pretreated with a BCRP inhibitor, elacridar, are useful as a non-rodent model. As model substances of BCRP substrates, SASP, RSV, FLV, and PTV were used for the present study. For these compounds, pharmacogenetic studies including *ABCG2* 421AA variants were conducted and reported for clinical data (8,12,13,19).

MATERIALS AND METHODS

Materials

RSV calcium and FLV sodium were purchased from Toronto Research Chemicals Inc. (North York, Canada). PTV calcium was obtained from AK Scientific Inc. (Mountain View, CA). Novobiocin sodium, rifampicin, and SASP were purchased from Sigma-Aldrich (St. Louis, MO). Elacridar was purchased from SynChem, Inc. (Elk Grove Village, IL). Cryopreserved cynomolgus monkey hepatocytes were obtained from BioreclamationIVT (Baltimore, MD). [^{3}H]RSV calcium (10 Ci/mmol), [^{3}H]PTV calcium (10 Ci/mmol), and

[^{3}H]FLV sodium (10 Ci/mmol) were obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). All other chemicals and reagents were of analytical grade and available from commercial resources.

Animals

Male Bcrp KO mice (FVB.129S6-Abcg2tm1Ahs) and wild type (WT) mice (FVB) at 6 weeks of age were obtained from Taconic Laboratories (Germantown, NY). They were housed in stainless steel cages for 7–14 days for acclimation. Male cynomolgus monkeys at 3–7 years of age were supplied by Gaoyao Kangda Laboratory Animal Science & Technology Co., Ltd. (Guangdong, China) and Guangxi Grandforest Scientific Primate Co., Ltd. (Guangxi, China). All animals were housed in a temperature- and humidity-controlled room with lighting at 12 h-intervals, fed a standard animal diet as appropriate for each species, and received water *ad libitum*. All experimental procedures were performed in accordance with the in-house guidelines of the Institutional Animal Care and Use Committee of Daiichi Sankyo Co., Ltd.

Pharmacokinetic Studies in Bcrp KO Mice and WT Mice

All the mice were fasted overnight with free access to water before drug administration. The body weights used for this study were 20 to 29 g. RSV, PTV, FLV, and SASP were administered intravenously *via* the tail vein or orally by gavages to mice at doses of 3, 1, 1, and 5 mg/kg, respectively. The dosing solutions of RSV, PTV, and FLV were prepared with 5% dimethylacetamide in saline, 1% lactic acid adjusted acceptable pH range by 1 M sodium hydrate, and 200 mM tris(hydroxymethyl)aminomethane, respectively. The formulations of SASP for intravenous (i.v.) and p.o. administration were a solution in 1% sodium hydrogen carbonate and a well triturated suspension in 0.5% aqueous methylcellulose, respectively. Blood samples were collected from the peripheral vein at 0.0833 (the group after i.v. only), 0.333, 1, 2, 4, 6, 8, and 24 h postdose under anesthesia. Plasma samples were separated by centrifugation (4°C, 21,900×*g*, 3 min), and stored at −20°C until analysis. Each group was comprised of 3 to 5 animals in these studies.

Pharmacokinetic Studies in Cynomolgus Monkeys with or Without Pretreatment of Elacridar

Monkeys were fasted overnight with free access to water before drug administration. The body weights used for this study were 3.2 to 5.9 kg. Ten minutes after p.o. administration of placebo or elacridar at the dose of 20 mg/kg as a well triturated suspension in 0.5% aqueous methylcellulose, RSV, PTV, FLV, and SASP were intravenously or orally administered *via* oral gavages at the dosages of 1, 1, 1, and 5 mg/kg, respectively. The formulations for the test compounds were the same as for the mice. Blood samples were collected at predose, 0.0833 (the group after i.v. only), 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h postdose. Plasma samples were separated by centrifugation (4°C, 21,900×*g*, 3 min), and stored at −20°C until analysis. Each group was comprised of 3 to 6 animals in these studies.

Efflux Transporter Inhibition Assays with Caco-2 Cells

Caco-2 cells were purchased from American Type Culture Collection (Manassas, VA; ATCC accession number CRL-2102). The culture period of Caco-2 cells for the efflux transporter inhibition assay was set as described previously (26). In brief, cells were grown in DMEM supplemented with 10% inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin in an atmosphere of 5% CO_2 at 37°C, and seeded on an HTS Transwell 24-well plate (Corning Inc., Corning, NY; 0.4 μm polycarbonate membrane; culture surface area 0.33 cm^2) at 5×10^4 cells/well for 15–17 days. The culture medium was changed every 3–4 days. On the day of the assay, the transepithelial electrical resistance (TEER) values in all the wells were measured to confirm the integrity of the monolayer. All the monolayers with TEER values greater than 300 $\Omega\times cm^2$ were used for the assay.

Before the assay, DMSO control, elacridar, and novobiocin were prepared by diluting the working solution to 1% (final concentration of elacridar, 0.16–100 μM; novobiocin, 100 μM) with the assay buffer (Hanks' balanced salt solution supplemented with 10 mM HEPES adjusted pH 7.4) containing each radiolabeled test compound ([^{3}H]RSV, [^{3}H]PTV, [^{3}H]FLV). The culture medium on both the apical and basal sides was changed with the assay buffer to wash the cells, and was then preincubated for 20 min at 37°C. The preincubated buffer on the apical or basal sides (donor side) was replaced with or without elacridar after that on the opposite side (receiver sides) was renewed by the assay buffer. At the same time, to assure BCRP inhibition in the assay, the vectorial transport of the test compounds was evaluated in the presence or absence of novobiocin. After 120 min incubation at 37°C, aliquots of the solutions were sampled from the receiver side and mixed with scintillation cocktails. The radioactivities were determined by a liquid scintillation counter. The counting was corrected by an external standard source method and the radioactivity in the samples was calculated by subtracting the background radioactivity from the observed radioactivity in the samples.

To evaluate the inhibition of SASP efflux by elacridar, the assay buffer containing 5 μM SASP was used instead of radiolabeled test compounds. After 120 min incubation at 37°C, aliquots of the solutions were also sampled from the

receiver side and stored at −20°C until quantification with LC/MS/MS.

Hepatocyte Uptake Assays Using Cynomolgus Monkey Hepatocytes

The procedure of hepatocyte uptake assay was described previously (27). In brief, cryopreserved cynomolgus monkey hepatocytes (Lot LEK, pool of 3 male monkeys) were thawed and resuspended to 2×10^6 viable cells/mL in 37°C prewarmed assay buffer. Hepatocyte viability was determined by trypan blue staining. The cell suspension was prewarmed at 37°C for 3 min until the start of the uptake transporter inhibition assays. The assays were then initiated by the addition of an equal volume of assay buffer containing radiolabeled compounds ([^{3}H]RSV, [^{3}H]PTV, or [^{3}H]FLV) with or without elacridar (final cell concentration: 1×10^6 cells/mL; final concentration of elacridar, 0.003–30 μM, respectively). At the same time, to assure that uptake transporters by OATPs inhibition work properly in the assay, the hepatic uptake transport of test compounds was evaluated in the presence or absence of rifampicin (final concentration, 100 μM). After the 37°C incubation (0.5 and 1 min), 60 μL aliquots of the cell mixtures were collected and then placed in a centrifuge tube containing 100 μL of oil (density, 1.015, a mixture of silicone-mineral oil; Sigma-Aldrich) on the top of 100 μL of 3 M potassium hydroxide solution to separate the cells from the transport buffer. The living cells were passed through the silicone-mineral oil layer by centrifugation at $10{,}000\times g$ for 10 s using a tabletop centrifuge (Beckman Microfuge E; Beckman Coulter) and then dissolved in potassium hydroxide solution overnight at room temperature. The radioactivity in both cells and media were determined by a liquid scintillation counter after mixing with a scintillation cocktail.

To examine the uptake of SASP, 100 μL of 5 M ammonium acetate containing the internal standard was used for the living cell separation instead of 3 M potassium hydroxide solution. The sample tubes were centrifuged and stored at −80°C until quantification. An aliquot was taken from the upper media portion and quenched in methanol. The cells were deactivated by sonication and mixing with methanol after being transferred from the centrifuge tube to a new tube. The samples from both the media and cell portions were measured by LC/MS/MS.

Analytical Methods

All the sample preparations were performed using protein precipitation by acetonitrile and/or methanol. Following mixing, the precipitant was removed by filtration using a Captiva 96-well filter plate (Agilent Technologies, Santa Clara, CA). The resulting supernatants were injected into the liquid chromatography/quadrupole tandem mass spectrometry (LC/MS/MS) systems consisting of an API 4000 (AB SCIEX, Framingham, MA) and an ACQUITY UPLC system (Waters Corporation, Milford, MA). The two mobile phases were used with the composition of a two-solvent pair: A, 5 mM ammonium acetate and 0.2% formic acid with 5% acetonitrile, and B, 5 mM ammonium acetate and 0.2% formic acid with 95% acetonitrile; C, 5 mM ammonium acetate with 5% acetonitrile, and D, 5 mM ammonium acetate with 95% acetonitrile. The gradient for RSV, PTV, and FLV analysis was as follows: solvent B was held at 0.1% for 0.10 min, linearly ramped from 0.10 to 50% in 0.10 min, ramped from 50.0 to 99.9% in 0.40 min, kept at 99.9% from 0.30 to 0.80 min, and then immediately brought back down to 0.1% for reequilibration. The gradient for SASP analysis was as follows: solvent D was held at 0.1% for 0.10 min, linearly ramped from 0.1 to 20.0% in 0.10 min, ramped from 20.0 to 99.9% in 1.45 min, kept at 99.9% in 0.15 min, and then immediately brought back down to 0.1% for reequilibration. All the analytes were chromatographically separated by an ACQUITY UPLC BEH C_{18} column 1.7 μm, 2.1 mm ID× 50 mm (Waters Corporation) at 50°C with a flow rate of 0.8 mL/min. LC/MS/MS analysis was carried out using multiple reaction monitoring transitions for each test compound by Analyst (version 1.5.1, AB SCIEX) to process the chromatographic data. The precursor and product ion (m/z) pairs of RSV, PTV, FLV, and SASP were: 482.5/258.5, 421.7/290.0, 411.4/280.0 (positive ion mode), and 397.2/197.3 (negative ion mode), respectively. The ion chromatograms were integrated and quantified based on analyte/internal standard peak-area ratios using the Analyst data system. As the internal standard for the analysis, phenacetin, warfarin or niflumic acid was used in positive ion mode, and furosemide, warfarin or niflumic acid was used in negative ion mode.

Pharmacokinetic Analysis

The PK parameters (AUC_{all} and $t_{1/2}$ for i.v. and p.o., C_{max} and t_{max} for p.o., and V_{dss} for i.v.) were estimated by noncompartmental analysis with Phoenix WinNonlin (Ver. 6.3, Pharsight Corporation, Mountain View, CA). The CL_{tot} was calculated as the dose divided by AUC_{all} after i.v. administration. The $t_{1/2}$ was calculated as $\ln 2/\lambda z$, where λz is the elimination rate constant (the slope of the regression line of several points in the elimination phase by the least squares method). If the adjusted square of the correlation coefficient was less than 0.75, $t_{1/2}$ was not calculated and was expressed as NC (not calculated) due to the judgment of low reliability for fitting. In these cases, V_{dss} were also expressed as NC.

Bioavailability (F) was calculated using AUC_{all} data *via* p.o. and i.v. administration. The ratios of each PK parameter were calculated (mice: Bcrp KO/WT, monkeys: elacridar pretreatment/untreatment). In the case of the systemic clearance influenced by Bcrp knockout, the AUC_{all} changes after p.o. administration ($AUC_{po,KO}/AUC_{po,WT}$) were corrected by the CL_{tot} changes after i.v. administration ($CL_{tot,KO}/CL_{tot,WT}$). They are expressed by following equation:

$$\frac{AUC_{po,KO}}{AUC_{po,WT}} \times \frac{CL_{tot,KO}}{CL_{tot,WT}} = \frac{AUC_{po,KO} \times Dose_{KO}}{AUC_{iv,KO}} \Big/ \frac{AUC_{po,WT} \times Dose_{WT}}{AUC_{iv,WT}}$$

$$= \frac{AUC_{po,KO}}{AUC_{iv,KO}} \Big/ \frac{AUC_{po,WT}}{AUC_{iv,WT}} = \frac{F_{KO}}{F_{WT}}$$

Based on this equation, this correction was introduced to the F ratio (F_{KO}/F_{WT}).

In vitro Data Analysis and IC_{50} Determination

In the efflux transporter inhibition assay, the apparent permeability coefficient (P_{app}), efflux ratio (*ER*), and remaining activity (% of control) (*RA*) were calculated for IC_{50} determination. The transported amount of each substrate across the monolayer was calculated by the value of the transported concentrations of the substrates multiplied by the volume, and P_{app} was calculated using Eq. 1:

$$P_{app} = (dQ/dt)/(A \times C_0) \quad (1)$$

where dQ/dt, A, and C_0 represent the amounts of the test substrates transported within a given time period, the surface area of monolayer, and the initial concentrations of the substrates, respectively. The *ER* and *RA* was calculated using Eqs. 2 and 3, respectively:

$$ER = P_{app,BtoA}/P_{app,AtoB} \quad (2)$$

$$RA = \frac{ER \text{ with inhibitor} - 1}{ER \text{ without inhibitor} - 1} \times 100 \quad (3)$$

Intrinsic clearance in the hepatocyte uptake assay ($CL_{int,\ hepatocyte\ uptake}$) ($\mu L/10^6$ cells/min) was determined by calculating the slope of the uptake volume (V_d) ($\mu L/10^6$ cells) between 0.5 and 1 min using Eq. 4:

$$CL_{int,\ hepatocyte\ uptake} = \frac{V_{d,1\,min} - V_{d,0.5\,min}}{1 - 0.5} \quad (4)$$

The IC_{50} was determined by fitting the data of each *RA* or intrinsic uptake clearance to an Inhibitory I_{max} Model with Phoenix WinNonlin.

$$E_i = E_0 - \frac{I_{max} \times C}{C + IC_{50}} \quad (5)$$

where C is the elacridar concentration, E_i, E_0, and I_{max} were the activity (the *RA* or the intrinsic uptake clearance) of each substrate measured at the given inhibitor concentration of elacridar, the activity without inhibitor, and the activity of each substrate caused by the maximum inhibition with elacridar subtracted from E_0, respectively.

Statistical Analysis

All the statistical analyses were performed using SAS system Release 9.2 (SAS Institute, Inc. Cary, NC). Statistical significances were assessed by applying the unpaired for PK parameters from the mouse *in vivo* study or paired two-tailed *t*-test for those from the monkey *in vivo* study. A p value <0.05 was considered statistically significant.

RESULTS

Plasma Concentration Profiles in Bcrp KO Mice and WT Mice

Plasma concentrations of SASP in Bcrp KO mice at each time point were much higher than those in WT mice just after i.v. administration (Fig. 1d). The CL_{tot} value of SASP in Bcrp KO mice was significantly lower than that in WT mice (Bcrp KO/WT of 0.055; Table I). By contrast, the plasma concentration-time profiles of RSV and FLV in Bcrp KO mice were comparable to WT mice (Fig. 1a and c). The CL_{tot} of RSV and FLV

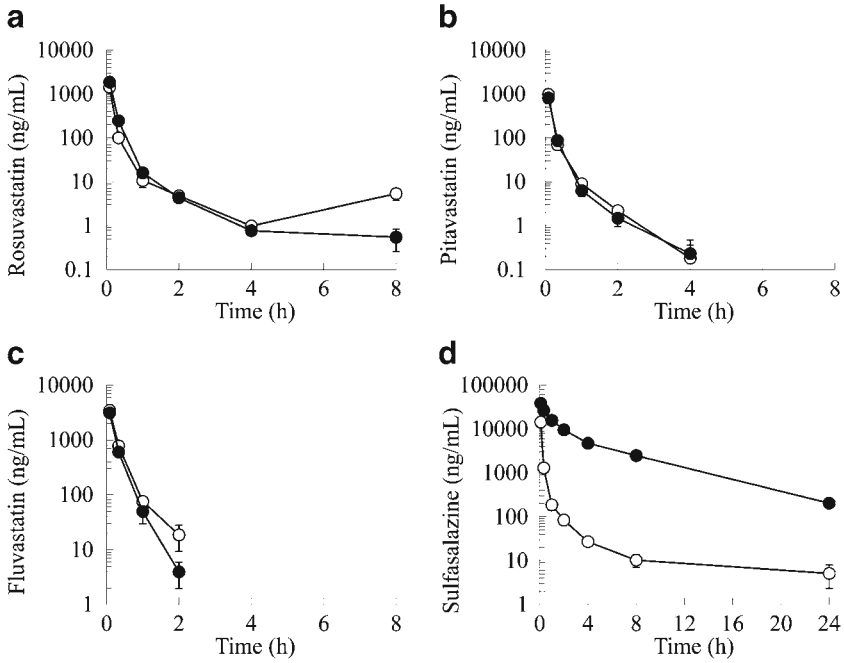

Fig. 1 Plasma concentration-time profiles after single intravenous administration to wild type (WT, ○) and Bcrp knockout (KO, ●) mice. (**a**) Rosuvastatin (3 mg/kg), (**b**) pitavastatin (1 mg/kg), (**c**) fluvastatin (1 mg/kg) and (**d**) sulfasalazine (5 mg/kg). Each *point* represents mean ± standard error of three mice in each group.

were almost the same between WT and Bcrp KO mice (Table I). As for PTV, although the effect was limited (Fig. 1b), KO mice tended to show higher CL_{tot} in comparison to WT mice (1.2-fold; Table I). After p.o. administrations, plasma concentrations of SASP in Bcrp KO mice at each time point were much higher than those in WT mice, in the same manner as in the case of i.v. administrations (Fig. 2d). In contrast, the exposures of RSV in Bcrp KO mice increased compared to those in WT mice, and those of PTV and FLV were comparable between Bcrp KO and WT mice, unlike i.v. administrations of PTV (Fig. 2a, b, and c). As shown in Table II, the AUC_{all} ratios (Bcrp KO/WT) of RSV, PTV, FLV, and SASP were 3.8, 1.2, 1.3, and 150, respectively, whereas the F ratios were 3.2, 1.5, 1.6, and 8.4, respectively. The F ratio of SASP was much lower than the AUC_{all} ratio of SASP, while the AUC_{all} ratios and F ratios of statins were

Table I Pharmacokinetic Parameters After Single Intravenous Administration of Rosuvastatin, Pitavastatin, Fluvastatin, and Sulfasalazine to Wild Type (WT) and Bcrp Knockout (KO) Mice

Parameter	WT	KO	KO/WT	WT	KO	KO/WT
	Rosuvastatin (3 mg/kg)			Pitavastatin (1 mg/kg)		
N	3	3	–	3	3	–
AUC_{all} (ng·h/mL)	499 ± 107	603 ± 98	1.2	306 ± 10	254 ± 11*	0.83
CL_{tot} (mL/min/kg)	110 ± 24	87.1 ± 13.2	0.79	54.6 ± 1.8	65.8 ± 2.7*	1.2
V_{dss} (L/kg)	NC	1.21 [a]	–	0.423 ± 0.040	0.572 ± 0.064	1.4
$t_{1/2}$ (h)	NC	0.674 [a]	–	0.489 ± 0.151	0.601 ± 0.325	1.2
	Fluvastatin (1 mg/kg)			Sulfasalazine (5 mg/kg)		
N	3	3	–	3	3	–
AUC_{all} (ng·h/mL)	1270 ± 80	1070 ± 40	0.84	4850 ± 630	88,600 ± 12,500**	18
CL_{tot} (mL/min/kg)	13.2 ± 0.8	15.6 ± 0.7	1.2	17.7 ± 2.1	0.976 ± 0.125**	0.055
V_{dss} (L/kg)	0.167 ± 0.022	0.158 ± 0.011	0.95	0.311 [a]	0.241 ± 0.023	0.77
$t_{1/2}$ (h)	0.246 ± 0.040	0.205 ± 0.030	0.83	1.40 [a)]	4.46 ± 0.19	3.2

Data are expressed as the mean ± standard error of three mice in each group

* $p < 0.05$ and ** $p < 0.01$, as compared to WT

NC not calculated; [a] $N = 1$

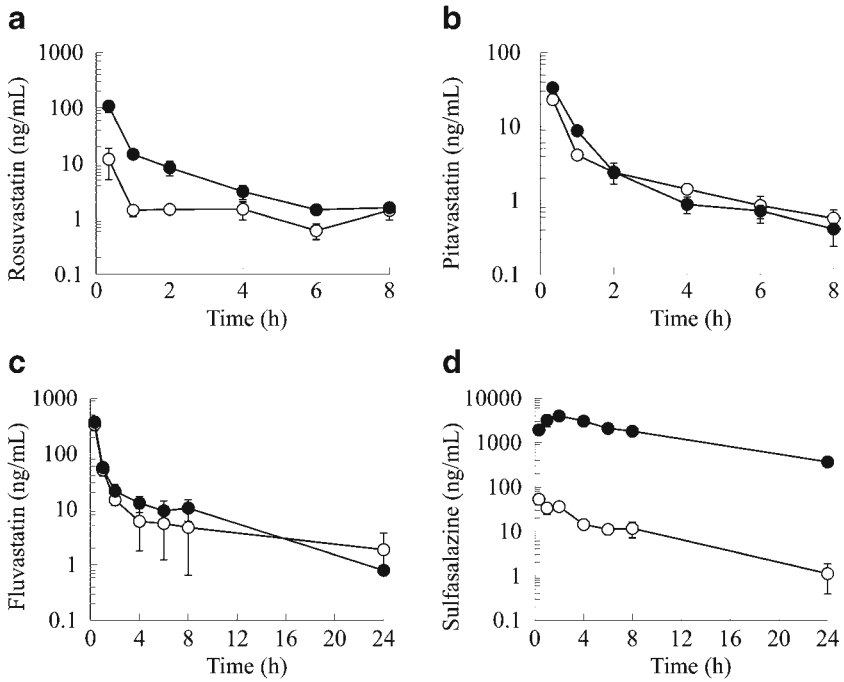

Fig. 2 Plasma concentration-time profiles after single oral administration to wild type (WT, ○) and Bcrp knockout (KO, ●) mice. (**a**) Rosuvastatin (3 mg/kg), (**b**) pitavastatin (1 mg/kg), (**c**) fluvastatin (1 mg/kg) and (**d**) sulfasalazine (5 mg/kg). Each *point* represents mean ± standard error of four to five mice in each group.

comparable. The C_{max} ratios of RSV, PTV, FLV, and SASP were 9.0, 1.4, 1.1, and 67, respectively. The t_{max} of statins were comparable between WT and Bcrp KO, however that of SASP in Bcrp KO mice were delayed approximately 3-times more compared to WT mice.

Plasma Concentration Profiles in Cynomolgus Monkeys With or Without Pretreatment of Elacridar

The plasma concentration-time profiles after single i.v. administration of RSV, PTV, FLV, and SASP to cynomolgus

Table II Pharmacokinetic Parameters After Single Oral Administration of Rosuvastatin, Pitavastatin, Fluvastatin, and Sulfasalazine to Wild Type (WT) and Bcrp Knockout (KO) Mice

Parameter	WT	KO	KO/WT	WT	KO	KO/WT
	Rosuvastatin (3 mg/kg)			Pitavastatin (1 mg/kg)		
N	5	5	–	5	5	–
AUC_{all} (ng·h/mL)	26.6 ± 5.1	102 ± 18**	3.8	28.4 ± 3.1	34.6 ± 2.1	1.2
$t_{1/2}$ (h)	3.21 [a]	2.86 ± 0.86 [d]	0.89	3.61 ± 0.77	2.10 ± 0.52 [d]	0.58
C_{max} (ng/mL)	12.0 ± 6.9	108 ± 26**	9.0	23.1 ± 3.1	33.4 ± 4.6	1.4
t_{max} (h)	0.333 ± 0.000	0.333 ± 0.000	1.0	0.333 ± 0.000	0.333 ± 0.000	1.0
Bioavailability (F,%)	5.33 ± 1.03	17.0 ± 3.0**	3.2	9.29 ± 1.01	13.6 ± 0.8*	1.5
	Fluvastatin (1 mg/kg)			Sulfasalazine (5 mg/kg)		
N	5	5	–	4	4	–
AUC_{all} (ng·h/mL)	313 ± 45	412 ± 66	1.3	256 ± 49	39,200 ± 3300***	150
$t_{1/2}$ (h)	9.45 ± 8.63 [c]	5.31 [b]	0.56	8.94 ± 3.56	7.68 ± 1.44	0.86
C_{max} (ng/mL)	335 ± 72	374 ± 62	1.1	61.2 ± 11.0	4110 ± 860**	67
t_{max} (h)	0.333 ± 0.000	0.333 ± 0.000	1.0	0.917 ± 0.394	2.50 ± 0.5*	2.7
Bioavailability (F,%)	24.6 ± 3.5	38.5 ± 6.2	1.6	5.27 ± 1.01	44.3 ± 3.7***	8.4

Data are expressed as the mean ± standard error of four to five mice in each group

*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, as compared to WT

NC not calculated; [a] $N = 1$, [b] $N = 2$, [c] $N = 3$, [d] $N = 4$

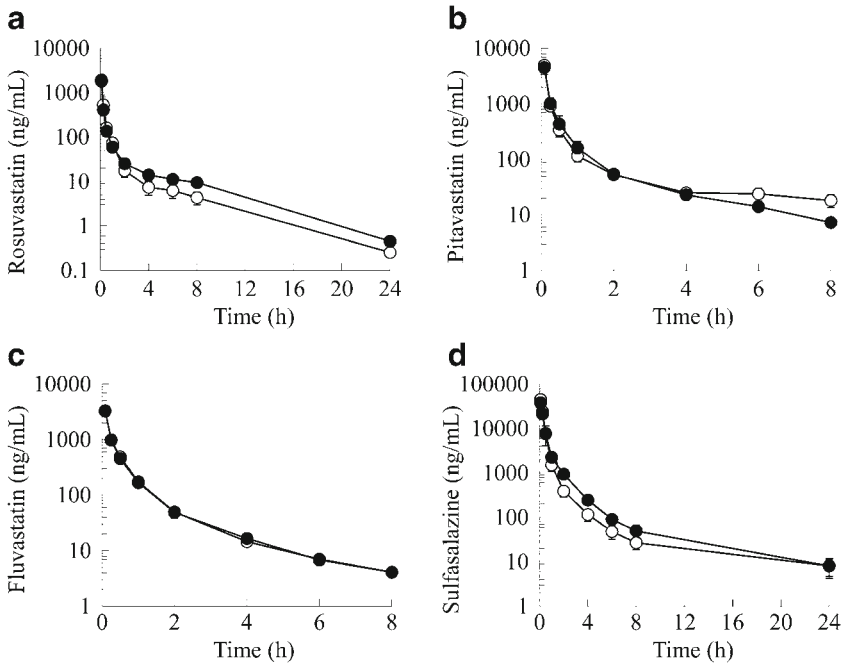

Fig. 3 Plasma concentration-time profiles after single intravenous administration to cynomolgus monkeys with elacridar (EL, ●) or untreatment (UT, ○). (**a**) Rosuvastatin (1 mg/kg), (**b**) pitavastatin (1 mg/kg), (**c**) fluvastatin (1 mg/kg) and (**d**) sulfasalazine (5 mg/kg). Each *point* represents mean ± standard error of three to six cynomolgus monkeys in each group.

monkey with elacridar pretreatment (EL) was comparable to that with untreatment (UT) (Fig. 3). The differences of CL_{tot} between EL and UT were within ±1.1-fold (Table III). Plasma concentration-time profiles after single p.o. administration of all the compounds tested on cynomolgus monkeys with EL or UT are shown in Fig. 4. The exposure of RSV, PTV, and SASP with EL increased compared to UT. As shown in Table IV, the AUC_{all} ratios (EL/UT) of RSV, PTV, and SASP were 3.1, 1.7, and 6.1, respectively, while the C_{max} ratios (EL/UT) of RSV, PTV, and SASP were 3.9, 4.3, and 4.0, respectively. On the contrary, no AUC_{all} and C_{max} changes of FLV were observed. The F ratios of all the compounds tested were comparable to AUC_{all} ratios after p.o. dosing. The t_{max} for FLV and SASP changed slightly with elacridar pretreatment, whereas those for RSV and PTV were 2.8 and 1.6-fold lengthened, respectively. However, the interindividual variations of t_{max} values for RSV and PTV were too large to confirm significant differences of this lengthening.

Relationships of AUC Ratios Between Mice (Bcrp KO/WT) and Human (*ABCG2* 421AA/CC)

Ratios of PK parameters calculated by human *ABCG2* 421 AA/CC after single p.o. administration of RSV, PTV, FLV,

Table III Pharmacokinetic Parameters After Single Intravenous Administrations of Rosuvastatin, Pitavastatin, Fluvastatin, and Sulfasalazine in Cynomolgus Monkeys With or Without Elacridar

Parameter	UT	EL	EL/UT	UT	EL	EL/UT
	Rosuvastatin (1 mg/kg, $N=3$)			Pitavastatin (1 mg/kg, $N=3$)		
AUC_{all} (ng·h/mL)	720 ± 101	747 ± 106	1.0 ± 0.0	1860 ± 270	1690 ± 400	0.90 ± 0.10
CL_{tot} (mL/min/kg)	24.0 ± 3.0	23.2 ± 3.0	0.96 ± 0.03	9.43 ± 1.50	11.0 ± 2.4	1.1 ± 0.1
V_{dss} (L/kg)	1.56 ± 0.58	2.43 ± 0.46	1.8 ± 0.3	0.356 [a]	0.210 ± 0.045	1.0 [a]
$t_{1/2}$ (h)	4.06 ± 0.25	3.86 ± 0.16	0.95 ± 0.04	4.55 [a]	2.27 ± 0.18	0.56 [a]
	Fluvastatin (1 mg/kg, $N=3$)			Sulfasalazine (5 mg/kg, $N=6$)		
AUC_{all} (ng·h/mL)	1330 ± 170	1330 ± 90	1.0 ± 0.1	18,600 ± 4800	18,700 ± 2300	1.2 ± 0.3
CL_{tot} (mL/min/kg)	12.9 ± 1.6	12.6 ± 0.8	0.99 ± 0.07	6.02 ± 1.27	4.82 ± 0.58	0.97 ± 0.17
V_{dss} (L/kg)	0.256 ± 0.011	0.241 ± 0.022	0.93 ± 0.05	0.175 ± 0.022	0.268 ± 0.041	1.6 ± 0.2
$t_{1/2}$ (h)	2.11 ± 0.06	1.73 ± 0.19	0.81 ± 0.10	5.77 ± 1.45	4.82 ± 0.88	1.1 ± 0.3

Data are expressed as the mean ± standard error of three to six cynomolgus monkeys in each group

There are no significant differences between UT and EL

UT: with pre-administration of 0.5% methylcellulose; EL: with pre-administration of elacridar at the dose of 20 mg/kg, [a] $N=2$

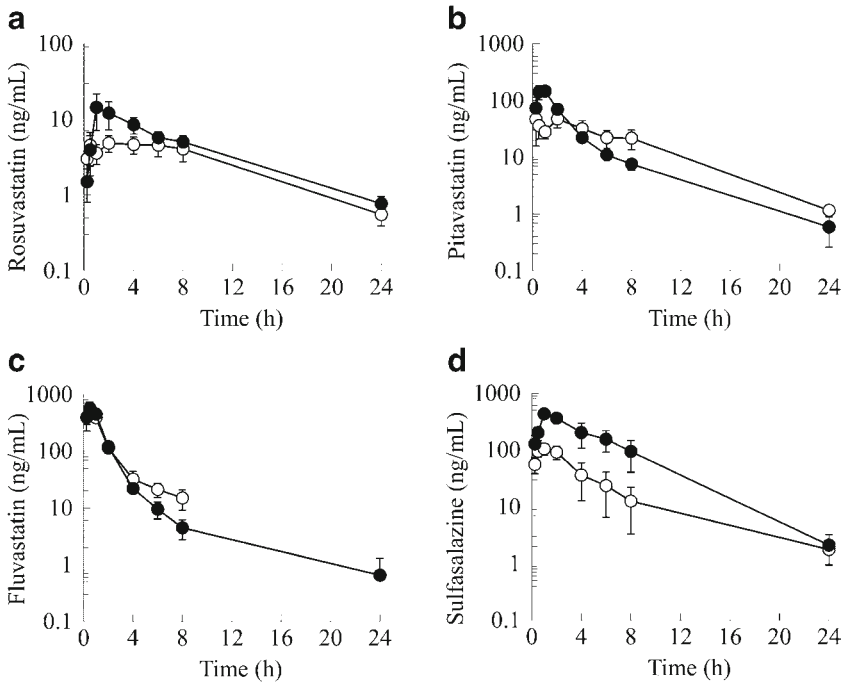

Fig. 4 Plasma concentration-time profiles after single oral administration to cynomolgus monkeys with elacridar (EL, ●) or untreatment (UT, ○). (**a**) Rosuvastatin (1 mg/kg), (**b**) pitavastatin (1 mg/kg), (**c**) fluvastatin (1 mg/kg) and (**d**) sulfasalazine (5 mg/kg). Each *point* represents mean ± standard error of six cynomolgus monkeys in each group.

and SASP from clinical data are summarized in Table V. The AUC_{all} ratios in mice (Bcrp KO/WT) were compared with those of human *ABCG2* 421AA/CC (Fig. 5a). The AUC ratios between mice and humans for statins were comparable. However, the AUC ratio in mice for SASP (150) was much higher than that in humans (3.5). To correct the effect to systemic clearance by Bcrp knockout for the AUC ratio after p.o. dosing, the F ratios (Bcrp KO/WT) were calculated and compared with the AUC ratios of humans (Fig. 5b). The F ratios between mice and humans for statins were still comparable. On the other hand, the F ratio in mice for SASP (8.4) was much closer to the human clinical AUC ratio (3.5) compared to the value without the correction (150).

Relationships of AUC Ratios Between Monkey (With or Without Elacridar) and Human (*ABCG2* 421AA/CC)

The AUC_{all} ratios in monkey (EL/UT) were compared with those of human *ABCG2* 421AA/CC as well (Fig. 6). The AUC_{all} ratios in monkeys were mostly within 2-fold

Table IV Pharmacokinetic Parameters After Single Oral Administrations of Rosuvastatin, Pitavastatin, Fluvastatin, and Sulfasalazine in Cynomolgus Monkeys With or Without Elacridar

Parameter	UT	EL	EL/UT	UT	EL	EL/UT
	Rosuvastatin (1 mg/kg, $N=6$)			Pitavastatin (1 mg/kg, $N=6$)		
AUC_{all} (ng·h/mL)	71.9 ± 18.7	111 ± 29	3.1 ± 1.8	430 ± 149	425 ± 70	1.7 ± 0.7
$t_{1/2}$ (h)	8.48 ± 1.97 [c]	6.67 ± 1.05	0.89 ± 0.04 [c]	4.73 ± 0.57	3.53 ± 0.72	0.81 ± 0.20
C_{max} (ng/mL)	7.52 ± 1.94	16.7 ± 6.9	3.9 ± 2.4	67.9 ± 27.9	167 ± 31*	4.3 ± 1.5
t_{max} (h)	2.54 ± 1.24	2.33 ± 0.56	2.8 ± 1.2	1.21 ± 0.36	1.00 ± 0.22	1.6 ± 0.6
Bioavailability (F,%)	9.99 ± 2.59	14.8 ± 3.8	3.1 ± 1.8	23.1 ± 8.0	25.2 ± 4.1	1.9 ± 0.8
	Fluvastatin (1 mg/kg, $N=6$)			Sulfasalazine (5 mg/kg, $N=6$)		
AUC_{all} (ng·h/mL)	1010 ± 150	939 ± 145	0.94 ± 0.07	511 ± 198	2550 ± 810*	6.1 ± 1.9
$t_{1/2}$ (h)	1.74 ± 0.29 [d]	1.40 ± 0.20 [b]	0.61 [a]	10.9 ± 8.8 [d]	2.70 ± 0.44 [d]	0.74 ± 0.23 [c]
C_{max} (ng/mL)	591 ± 89	714 ± 152	1.3 ± 0.3	136 ± 27	446 ± 61**	4.0 ± 0.9
t_{max} (h)	0.667 ± 0.105	0.708 ± 0.136	1.2 ± 0.3	1.25 ± 0.25	1.17 ± 0.17	1.2 ± 0.3
Bioavailability (F,%)	75.9 ± 11.4	70.6 ± 10.9	0.94 ± 0.07	2.75 ± 1.07	13.7 ± 4.4*	6.1 ± 1.9

Data are expressed as the mean ± standard error of six cynomolgus monkeys in each group. * $p<0.05$ and ** $p<0.01$, as compared to UT

UT: with pre-administration of 0.5% methylcellulose; EL: with pre-administration of elacridar at the dose of 20 mg/kg, [a] $N=2$, [b] $N=3$, [c] $N=4$, [d] $N=5$

Table V Mean Ratios of Pharmacokinetic Parameters After Single Oral Administrations of Rosuvastatin, Pitavastatin, Fluvastatin, and Sulfasalazine from Clinical Data Calculated by *ABCG2* 421 AA/CC

	Subjects of *ABCG2* 421 AA/CC	AUC	C_{max}	t_{max}	$t_{1/2}$	Reference
Rosuvastatin	*N* = 4/*N* = 16	2.4 [a]	2.3	0.80	0.97	(12)
Pitavastatin	*N* = 3/*N* = 11	0.96 [b]	1.3	–	1.2	(19)
Fluvastatin	*N* = 5/*N* = 23	1.7 [b]	1.9	0.50	1.2	(13)
Sulfasalazine	*N* = 9/*N* = 12	3.5 [b]	2.6	0.91	1.1	(8)

[a] AUC from 0 h to infinity

[b] AUC from 0 h to last observed time point

differences from clinically observed AUC ratios in human *ABCG2* 421AA/CC.

Inhibitory Effect of Elacridar on the Efflux Transporter Using Caco-2 Cells

The IC_{50} values of elacridar in the efflux transport of RSV, PTV, FLV, and SASP were calculated by the efflux transporter inhibition assays using Caco-2 cells (Table VI). The Caco-2 efflux of all the test compounds was inhibited by elacridar in a dose-dependent manner, which was also reduced by novobiocin, a potent inhibitor of BCRP (data not shown). The IC_{50} values of elacridar for RSV, PTV, FLV, and SASP were 1.78, 0.601, 0.830, and 1.16 μM, respectively.

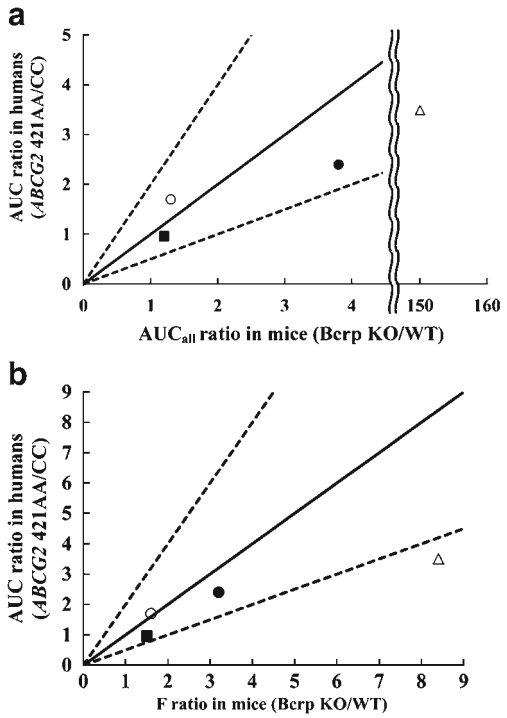

Fig. 5 Relationships of (A) the AUC_{all} ratio or (B) the F ratio between mice (Bcrp knockout (KO)/wild type (WT)) and human (*ABCG2* 421AA/CC) after oral administration of rosuvastatin (●), pitavastatin (■), fluvastatin (○), and sulfasalazine (Δ). The *solid lines* represent a 1:1 correspondence and the *dotted lines* represent the 2-fold difference.

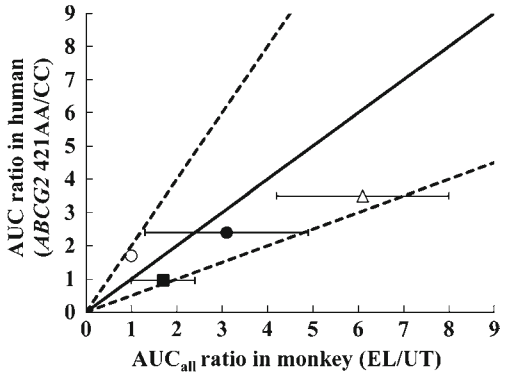

Fig. 6 Relationships of the AUC ratio between monkeys (with elacridar (EL) / untreatment (UT)) and human (*ABCG2* 421AA/CC) after oral administration of rosuvastatin (●), pitavastatin (■), fluvastatin (○), and sulfasalazine (Δ). The *solid lines* represent a 1:1 correspondence and the *dotted lines* represent the 2-fold difference. Data are expressed as the mean ± standard error of six cynomolgus monkeys in each group.

Inhibitory Effect of Elacridar on the Uptake into Cynomolgus Monkey Hepatocytes

The IC_{50} values of elacridar in the hepatic uptake of RSV, PTV, FLV, and SASP were calculated by hepatocyte uptake assay using cryopreserved cynomolgus monkey hepatocytes (Table VI). The hepatic uptake assay directly measures the cellular uptake of investigational drugs, thereby directly determining hepatic active uptake clearance. The cellular uptake of all the test compounds was inhibited by elacridar in a dose-dependent manner, which was also attenuated by rifampicin, a potent inhibitor of OATPs (data not shown). The IC_{50} values of elacridar for RSV, PTV, FLV, and SASP were 1.88, 0.891, 0.345, and 1.33 μM, respectively.

DISCUSSION

The purpose of the present study is to elucidate whether the impact of functional modulation of BCRP on the gastrointestinal disposition of its substrates observed in *ABCG2* 421C>A

Table VI IC_{50} Values of Elacridar for Rosuvastatin, Pitavastatin, Fluvastatin, and Sulfasalazine Obtained in the Efflux Transporter Inhibition Assays Using Caco-2 Cells and in the Uptake Transporter Inhibition Assays Using Cryopreserved Monkey Hepatocytes

	Efflux transporter inhibition IC_{50} (μM)	Uptake transporter inhibition IC_{50} (μM)
Rosuvastatin	1.78	1.88
Pitavastatin	0.601	0.891
Fluvastatin	0.830	0.345
Sulfasalazine	1.16	1.33

subjects can be assessed by pre-clinical studies using Bcrp KO mice or cynomolgus monkeys with pretreatment with a BCRP inhibitor. Using well-known BCRP substrates (RSV, PTV, FLV, and SASP), the correlation of AUC changes caused by *ABCG2* 421C>A with those by knockout of Bcrp in mice was investigated. In parallel, such investigations were carried out using monkeys, which are widely used for human PK prediction (28,29), where elacridar was used as the inhibitor of BCRP.

The metabolism and excretion of the BCRP substrates selected in this study were known to determine the PK in mice, monkeys, and humans. RSV is hardly metabolized *in vivo* (30,31). The metabolism of PTV by cytochrome P450 (CYP) enzymes in humans was limited and was less than in monkeys (30,32,33). FLV is extensively metabolized by the first-pass effect in mice, monkeys, and humans with CYP2C (30,33). SASP is low-permeable and mainly degraded by intestinal bacteria in mice and humans, not by CYPs (34,35). With regard to transporters, these statins are also well-known substrates of human OATPs (1B1, 1B3, and 2B1) besides BCRP, mainly uptaken to the liver *via* OATP1B1, and excreted into the bile (30). SASP is known as the human OATPs inhibitor (36), however the contribution of these uptake transporters on PK remains unknown. As for renal clearance, low urinary excretions of PTV and FLV were observed in humans and animals (30,32,33). The renal clearance of RSV in humans after oral dosing was unaffected by the *ABCG2* genotype (12), although it occupied approximately 30% of total clearance (31). Species difference in SASP was reported. Renal clearance was estimated to account for 37% of total clearance in humans (37), whereas that in rats and dogs was limited (38). Although a variety of factors (CYPs and transporters) can affect the PK of BCRP substrates as described above, the functional modulation of BCRP in the absorption phase is considered to be adequately assessable *in vivo* by comparing control animals with BCRP-defected animals after oral administration of BCRP substrates, as long as one of the following two conditions is satisfied. First, the BCRP substrates undergo no metabolism or active transport in BCRP-defected animals, or, if any, only slightly. Second, even if the BCRP substrates are susceptible to metabolism or active transport, they correct the effect on clearance by intravenous administration.

In mice, the AUC_{all} of SASP after i.v. and p.o. administrations in Bcrp KO mice was drastically elevated compared to WT mice (AUC_{all} ratio: 18 for i.v., 150 for p.o.; Figs. 1 and 2, Tables I and II). The CL_{tot} of SASP in Bcrp KO mice was significantly lower compared to WT mice (CL_{tot} ratio: 0.055; Fig. 1, Table I), suggesting that Bcrp-mediated pathways, such as biliary excretion, mainly contributed to the systemic clearance observed in the study using Bcrp KO rats (39). Thus, the marked AUC_{all} elevation of SASP in Bcrp KO mice after p.o. dosing may be attributed to the fact that Bcrp knockout produced an additive effect on the increase of the intestinal absorption as well as the decrease of the Bcrp-mediated systemic clearance in Bcrp KO mice. In contrast, the increase of AUC_{all} ratios of RSV after p.o. administration may be exclusively accounted for by the increase of absorption *via* Bcrp knockout, because CL_{tot} in Bcrp KO mice was almost comparable to WT mice. In PTV, PK parameters showed no differences between Bcrp KO and WT mice after p.o. administrations, consistent with the report by Ieiri *et al.* (19), whereas the marginally significant increase in CL_{tot} was observed in KO mice by i.v. administration (1.2-fold) for unknown reasons. Although the reduced biliary excretion clearance of PTV in Bcrp KO mice compared to WT mice was reported by Hirano *et al.* (6), the minor contribution of biliary excretion clearance *via* Bcrp to systemic clearance for PTV would explain the limited PK changes of PTV in Bcrp KO mice compared to WT mice. As for FLV, the impact of Bcrp knockout on mice PK was limited.

The correlation of AUC changes caused by human *ABCG2* 421C>A with those by knockout of Bcrp in mice was examined. The AUC_{all} ratio of SASP (150) in mice (Bcrp KO/WT) was much higher compared to that in human *ABCG2* 421AA/CC (3.5), although the AUC_{all} ratios of RSV (3.8), PTV (1.2), and FLV (1.3) in Bcrp KO mice were comparable to those in human *ABCG2* 421AA/CC (RSV: 2.4, PTV: 0.96, FLV: 1.7; Fig. 5, Tables II and V). As previously mentioned, in humans, the AUC increase of SASP observed in *ABCG2* 421C>A subjects is considered to be mainly caused by the increase of absorption, since $t_{1/2}$ and t_{max} of SASP was not significantly affected by *ABCG2* 421C>A (8). In contrast, in mice, the existence of Bcrp-mediated excretion pathways such as biliary and urinary excretion and direct intestinal secretion also contributed to the systemic clearance (Fig. 1, Table I), which may explain the higher AUC ratio of SASP in mice compared to human *ABCG2* 421AA/CC. Thus, in order to minimize the effect on systemic clearance by Bcrp knockout, the F ratio, the AUC changes after p.o. administration corrected by the CL_{tot} changes after i.v. administration, was introduced. By the correction of the F ratio, the AUC ratio of SASP, which showed a large difference in mice without correction (150), was calculated to be 8.4, which is closer to the AUC ratio in humans (3.5). The AUC ratios of statins in mice were in good agreement with those in humans regardless of the correction of the F ratio, since the systemic clearance was only slightly affected by Bcrp knockout. Consequently, the effect on the absorption by *ABCG2* 421C>A variants in humans is considered to be successfully evaluated by using Bcrp KO mice with introduction of the F ratio.

In monkeys, AUC changes caused by the functional decrease of BCRP were examined using elacridar. Elacridar was selected as the *in vivo* BCRP inhibitor since pretreatment with elacridar improved oral bioavailability of topotecan in humans by BCRP inhibition (40,41). The oral predose of

elacridar, which was set at 20 mg/kg throughout this study was considered to be sufficient to achieve the maximum AUC increase of SASP in monkeys, in relation to the elacridar concentration in the gut (elacridar dose (20 mg/kg) / 250 mL) which is high enough to reach IC_{50} values of elacridar for RSV, PTV, FLV, and SASP in Caco-2 cells (Table VI). Still, the possibility remains that 20 mg/kg of elacridar pretreatment affects the PK changes of these test substrates by means other than BCRP inhibition through the absorption phase to the systemic circulation, and inhibitory effects on hepatic uptake transporters and CYPs were investigated. Although strong inhibitory effects on hepatocyte uptake of RSV, PTV, FLV, and SASP by elacridar were observed (Table VI), calculated R-values ($=1+(f_u \times I_{in,max}/IC_{50})$) were approximately 1.0, where f_u and $I_{in,max}$ are used from the reported values of monkey plasma protein binding and portal C_{max} of elacridar (0.001 and 985 ± 315 ng/mL at 30 mg/kg suspension, respectively; (22)), suggesting that inhibition of these substrates on uptake transporters *in vivo* is minimal. Also for CYPs, 20 mg/kg of elacridar treatment was considered not to inhibit the metabolism by CYPs in monkey liver in view of *in vitro* inhibition studies for human CYPs (22). This is further supported by the observance of no clearance change of antipyrine in intravenous monkey PK with/without elacridar predosing (data not shown). Taken together, the oral pretreatment by elacridar at 20 mg/kg was considered to work for the purpose of evaluating the influence of the absorption *via* BCRP.

The AUC ratio of statins and SASP in monkeys with or without pretreatment of elacridar at 20 mg/kg was investigated. Elacridar pretreatment did not affect the BCRP-mediated systemic clearance of all the compounds tested in monkeys based on the observation after a single i.v. administration (Fig. 3 and Table III). Under these conditions, the AUC ratio after oral administration is nearly equal to the F ratio and also considered to be an F_a (fraction absorbed) ratio because the F_g (bioavailability in the gut) and F_h (bioavailability in the liver) of all the compounds tested are unchanged with/without elacridar pretreatment. The AUC_{all} of SASP after p.o. dosing to monkeys was significantly elevated with pretreatment by elacridar (Fig. 4 and Table IV). The AUC_{all} ratio (EL/UT) of SASP in monkeys (6.1) was less than the F ratio (8.4) in mice (Bcrp KO/WT) and closer to the AUC ratio in humans (3.5). The oral exposure of RSV in monkeys was also increased by elacridar. As for FLV, influence of elacridar pretreatment on PK was limited in monkeys. On the other hand, the AUC_{all} and C_{max} of PTV after p.o. dosing to monkeys were increased by elacridar predose, although the PK profiles of PTV in Bcrp KO and WT mice were comparable after oral administration. This might be explained by the fact that elacridar pretreatment also inhibited P-gp, because PTV was also known as a P-gp substrate (6,32) and the IC_{50} of elacridar for P-gp was lower than that of BCRP (42).

In terms of the estimation of the clinical impact of BCRP modulation, the AUC_{all} ratios of all the compounds tested in monkeys (EL/UT) were comparable to those in human *ABCG2* 421AA/CC within approximately 2-fold ranges, indicating that monkey PK studies with a BCRP inhibitor are useful as a non-rodent model when assessing how BCRP impacts on the gastrointestinal absorption in humans.

CONCLUSION

In conclusion, the studies using Bcrp KO mice serve as an estimation of the clinical AUC changes in *ABCG2* 421C>A variants for BCRP substrate drugs with the F ratio correcting the systemic clearance change by Bcrp knockout in Bcrp KO mice. As a non-rodent model, the studies using cynomolgus monkeys with elacridar were investigated and were also confirmed to be useful for evaluation of the absorptional function *via* BCRP. Further studies for the discovery of more selective BCRP inhibitors instead of elacridar are required to assess the BCRP function adequately in monkeys since elacridar has the issue of P-gp inhibition. This study contributes not only to the evaluation of the impact of functional alteration of BCRP on the PK of investigational drugs in humans but also to the estimation of drug-drug interaction potential *via* BCRP.

ACKNOWLEDGMENTS AND DISCLOSURES

The authors acknowledge Norio Suzuki, Kenichi Itokawa, and Rie Komura for technical assistance.

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