DMD Fast Forward. Published on January 24, 2006 as doi:10.1124/dmd.105.008219

Breast Cancer Resistance Protein (BCRP/ABCG2) transports fluoroquinolone antibiotics and affects their oral availability, pharmacokinetics and milk secretion

Gracia Merino, Ana I. Álvarez, Mivis M. Pulido, Antonio J. Molina, Alfred H. Schinkel and Julio G. Prieto

University of León, Department of Physiology, Faculty of Veterinary Medicine, Campus de Vegazana 24071 León, Spain (G.M., A.I.A, M.M.P., A.J.M., J.G.P)

The Netherlands Cancer Institute, Division of Experimental Therapy, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands (A.H.S)

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: Bcrp1 affects disposition and milk secretion of quinolones

Corresponding author:

Julio G. Prieto,

Department of Physiology,

Faculty of Veterinary Medicine, University of Léon,

Campus de Vegazana,

24071 León, Spain.

Telephone: +34-987291263.

FAX: +34-987291267.

E-mail: dfijpf@unileon.es

Number of text pages: 24

Number of tables: 0

Number of figures: 6

References: 37

Number of words in the Abstract: 214

Number of words in the Introduction: 626

Number of words in the Discussion: 834

ABBREVIATIONS: AUC, area under the plasma concentration-time curve; BCRP, breast cancer resistance protein; HPLC, high performance liquid chromatography; Ko143, 3-(6-isobutyl-9-methoxy-1,4-dioxo-1,2,3,4,6,7,12,12a-octahydropyrazino[1´,2´:1,6]pyrido[3,4-b]indol-3-yl]-propionic acid *tert*-butyl ester; MDCK, Madin-Darby canine kidney; MF, median of fluorescence; MRP2, multidrug-resistance-associated protein 2; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Abstract

The Breast Cancer Resistance Protein (BCRP/ABCG2) is an ATP binding cassette drug efflux transporter that extrudes xenotoxins from cells in intestine, liver, mammary gland and other organs, affecting the pharmacological and toxicological behavior of many compounds, including their secretion into the milk. The purpose of this study was to determine whether three widely used fluoroquinolone antibiotics (ciprofloxacin, ofloxacin and norfloxacin) are substrates of Bcrp1/BCRP and to investigate the possible role of this transporter in the *in vivo* pharmacokinetic profile of these compounds and their secretion into the milk. Using polarized cell lines, we found that ciprofloxacin, ofloxacin and norfloxacin are transported by mouse Bcrp1 and human BCRP. *In vivo* pharmacokinetic studies showed that ciprofloxacin plasma concentration was more than 2-fold increased in Bcrp1$^{-/-}$ compared to wild-type mice (1.77 ± 0.73 *versus* 0.85 ± 0.39 μg/ml, $p < 0.01$) after oral administration of ciprofloxacin (10 mg/kg). The AUC in Bcrp1$^{-/-}$ mice was 1.5-fold higher than that in wild-type mice (48.63 ± 5.66 *versus* 33.10 ± 4.68 min.μg/ml, $p < 0.05$) after i.v. administration (10 mg/kg). The milk concentration and milk to plasma ratio of ciprofloxacin was 2-fold higher in wild-type compared to Bcrp1$^{-/-}$ lactating mice. We conclude that Bcrp1 is one of the determinants for the bioavailability of fluoroquinolones and their secretion into the milk.

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Quinolone antimicrobial drugs are widely used due to their broad spectrum and intense bactericidal activity. They are developed for oral and parenteral use in the treatment of bacterial diseases, including severe systemic infections (Brunner and Zeiler, 1988). Most quinolone antibacterial drugs are rapidly absorbed from the intestine, with a bioavailability of close to 90%, and then penetrate well into most body tissues and fluids. However, some fluoroquinolones have been reported to undergo efflux, which can explain the low bioavailability of some of them; for instance, the bioavailabilities of ciprofloxacin and norfloxacin are 50-80% and 30-40%, respectively (Sörgel et al., 1989; Lamp et al., 1992). In addition, at least 10% of i.v. administered ciprofloxacin is eliminated by intestinal secretion (Rohwedder et al., 1990). Only 1% of the dose is excreted into the bile (Parry et al., 1988). Some studies indicated that intestinal elimination of ciprofloxacin was not mediated by P-glycoprotein (ABCB1) (Griffiths et al., 1993; Cavet et el., 1997; Dautrey et al., 1999), one of the most important members of the ATP-binding cassette group of transporters, and often involved in restricting the bioavailability of drugs. Accordingly, several groups (Lowes and Simmons, 2002; Michot et al., 2004) have recently shown that ciprofloxacin is not a substrate of this transporter nor of MRP2 (ABCC2), another ABC transporter. However, the pharmacokinetics of ciprofloxacin was suggested to involve one or more active intestinal or hepatobiliary transport mechanisms in rats (Dautrey et al., 1999). Norfloxacin and ofloxacin have also been shown to be subject to active efflux (Cao et al., 1992; Rabaa et al., 1996). Active secretory mechanisms common to all fluoroquinolones have been suggested (Griffiths et al. 1993, 1994), as well as competition between fluoroquinolones at transporter sites (Rabaa et al., 1996). However, the precise mechanisms involved in the pharmacokinetics of ciprofloxacin and other fluoroquinolones remain to be clarified.

Several adverse effects have been reported with the use of quinolones, including nausea, diarrhea, vomiting, dizziness, headache, insomnia and phototoxicity (Stahlmann, 2002). Arthropathy (condrotoxicity, tendopathies) in immature animals has been observed after administration of all quinolones tested so far. Although the significance of this undesirable side effect for humans remains unclear, the toxic potential has led to the contraindication of quinolones in children and women during pregnancy and lactation. Despite the extensive use of fluoroquinolones, little is known about their secretion into human breast milk. More accurate data on the passage of these compounds into breast milk will allow for the establishment of better recommendations for their use in lactating women (Dan et al., 1993).

Breast Cancer Resistance Protein (BCRP/ABCG2), another member of the ABC family of transporters, is apically expressed and mediates the active and outward transport of a range of anticancer drugs, dietary compounds, food carcinogens and antibiotics (van Herwaarden et al., 2003; Burger et al., 2004; Merino et al., 2005a). BCRP is found not only in tumor cells but also in a variety of normal tissues such as intestine, liver, brain and mammary gland. Several *in vivo* studies indicated that Bcrp1 mediates the hepatobiliary excretion of its substrates and limits their oral bioavailability and fetal penetration (van Herwaarden et al., 2003; Jonker et al., 2000). Induced expression of Bcrp1/ BCRP in the lactating mammary gland and an important role of this ABC transporter in the active secretion of several xenobiotics into milk have been demonstrated (Jonker et al., 2005). Since fluoroquinolone breast milk concentrations are higher than serum concentrations (Giamarellou et al., 1989), we hypothesized that the pharmacokinetics and milk secretion of these fluoroquinolones could be regulated by BCRP *in vivo*.

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

In this study, we have demonstrated that three fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin) are transported *in vitro* by Bcrp1/BCRP using polarized cell lines and that this transport results in altered pharmacokinetics and milk secretion of ciprofloxacin in Bcrp1$^{-/-}$ mice.

Material and Methods

Animals. Mice were housed and handled according to institutional guidelines complying with European legislation. Animals used were male or lactating female Bcrp1$^{-/-}$ and wild-type mice, all of >99% FVB genetic background between 9 and 15 weeks of age. Animals were kept in a temperature-controlled environment with a 12-h light/12-h dark cycle and received a standard diet (Panlab; Barcelona, Spain) and water *ad libitum*.

Chemicals. Ofloxacin, norfloxacin and oxfendazole were from Sigma Chemical Co. (Steinheim, Germany); Ciprofloxacin was from Fluka Chemie (Buchs, Switzerland); isoflurane (Isovet®) was from Schering-Plough (Madrid, Spain); oxytocin (Oxiton®) was from Ovejero (León, Spain); Ko143 was described previously (Allen et al., 2002). All other compounds used were reagent grade.

Cells and tissue culture. The polarized canine kidney cell line MDCK-II was used in the transport assays. Human BCRP- and murine Bcrp1-transduced MDCK-II subclones were described previously (Jonker et al., 2000; Pavek et al., 2004). The cells were cultured in DMEM supplied with glutamax (Life Technologies, Inc.) and supplemented with penicillin (50 units/ml), streptomycin (50 μg/ml), and 10% (v/v) fetal calf serum (MP Biomedicals) at 37ºC in the presence of 5% CO_2. The cells were trypsinized every 3 to 4 days for subculturing.

Transport assays. Transport assays were carried out as described earlier (Merino et al., 2005a), with minor modifications. Cells were seeded on microporous membrane filters (3.0 μm pore size, 24 mm diameter; Transwell 3414; Costar, Corning, NY) at a density of 1.0 x 10^6 cells per well. Cells were grown for 3 days, and medium was replaced every day. Transepithelial resistance was measured in each well using a Millicell ERS ohmmeter (Millipore, Bedford, MA); wells registering a resistance of 200

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

ohms or greater, after correcting for the resistance obtained in control blank wells, were used in the transport experiments. The measurement was repeated at the end of experiment to check the tightness of the monolayer. Two hours before the start of the experiment, medium at both the apical and basolateral side of the monolayer was replaced with 2 ml of Optimem medium (Life Technologies, Inc.), without serum, either with or without 1 μM Ko143. The experiment was started (t = 0) by replacing the medium in either the apical or basolateral compartment with fresh Optimem medium, either with or without 1 μM Ko143 and containing 10 μM fluoroquinolone. Cells were incubated at 37ºC in 5% CO_2 and 100-μl aliquots were taken at t = 2 and 4 hours, and stored at –20ºC until the time of analysis. The appearance of fluoroquinolone in the opposite compartment was measured by HPLC as described below, and presented as the fraction of total fluoroquinolone added at the beginning of the experiment.

Accumulation Assays. *In vitro* accumulation assays were carried out as described previously (Pavek et al., 2005). In brief, 5 μM mitoxantrone was used as fluorescent substrate and fluoroquinolones were used as inhibitors at different concentrations. Cells were cultured in 12-well plates (20×10^3 cells/well) in complete medium for 36 hours to subconfluence. Medium was aspirated, and cells were preincubated in prewarmed Optimem medium with or without fluoroquinolone for 60 min before adding 5 μM mitoxantrone. Accumulation of mitoxantrone was allowed for 1 hour at 37°C. Cells were washed with ice-cold PBS and trypsinized. Collected cells were sedimented and resuspended in PBS with 2.5% of fetal calf serum. Relative cellular accumulation of mitoxantrone was determined by flow cytometry using a FACSCalibur cytometer. Excitation and emission wavelengths for mitoxantrone were 488 nm and 650 nm, respectively. Fluorescence of accumulated substrate in tested populations of at least 5,000 cells was quantified from histogram plots using the median of fluorescence (MF).

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

BCRP inhibition increases accumulation of mitoxantrone in Bcrp1/BCRP-transduced cells and thus increases MF. Possible background fluorescence of all tested fluoroquinolones was checked in appropriate channels, but the fluorescence was negligible in all cases. Flow cytometry data were processed and analyzed using WinMDI ver.2.8 software.

Pharmacokinetic experiments. For oral administration of ciprofloxacin (10 mg/kg), 10 μl of drug solution (appropriate concentration in 5% glucose and 1% NaOH 1M)/g body weight were dosed by gavage into the stomach. For i.v. administration of ciprofloxacin (10 mg/kg), 5 μl of drug solution (appropriate concentration in 5% glucose and 1% NaOH 1M)/g body weight were injected into the tail of mice lightly anesthesized with isoflurane. Blood was collected by orbital bleeding after anesthesia with isoflurane. Heparinized blood samples were centrifuged immediately at 3000 x g for 15 min and plasma collected and stored at –20ºC until the time of HPLC analysis. 4-9 animals were used for each time point.

Milk secretion experiments. For milk experiments, pups of approximately 10 days old were separated from the mother approximately 4 hours before starting the experiment. To stimulate milk secretion, oxytocin (200 μl of 1 I.U./ml solution) was administered subcutaneously to lactating dams. Ciprofloxacin (5 mg/kg) was injected into the tail vein, and milk was collected at t = 10 min from the mammary glands by gentle vacuum suction. Blood samples were collected by orbital bleeding under anesthesia with isoflurane. Milk and plasma were stored at –20ºC until the time of HPLC analysis. 3 animals were used for each group.

HPLC analysis. The conditions for HPLC analysis of quinolones were modified based on previously published methods (Idowu et al., 2004; Marazuela et al., 2004). To each 100-μl aliquot of sample, 10 μl of a 12.5 μg/ml oxfendazole solution were added

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

as an internal standard in a 1.5 ml reaction tube. The mixture was vortexed vigorously, and 300 µl of dichloromethane were added. Samples were shaken for 60 s and the organic and water phases were separated by centrifugation at 5000 x g for 5 min and evaporated to dryness under a nitrogen stream. The samples were resuspended in 100 µl of methanol and injected into the HPLC system. Samples from the transport assays were not processed and 100 µl of the culture media were directly injected into the HPLC system. The system consisted of a Waters 600 pump, a Waters 717plus autosampler and a Waters 2487 UV detector. Separation of the samples was performed on a reversed-phase column (AQUA^{TM} C18 polar encapped, 5-µm particle size, 250 mm x 4.6 mm). The composition of the mobile phase was 25 mM orthophosphoric acid (pH 3.0)/acetonitrile (77:23). The flow rate of the mobile phase was set to 1.0 ml/min. UV absorbance was measured at 278 nm. Peak area ratios (fluoroquinolone/oxfendazole) were used for comparison with the standard curve. The integration was performed using the software Millennium^{32} (Waters). Standard samples in the appropriate drug-free matrix were prepared yielding a concentration range from 0.08 µg/ml up to 10 µg/ml.

Pharmacokinetic calculations and statistical analysis. The two-sided unpaired Student's *t* test was used throughout to assess the statistical significance of differences between the two sets of data. Results are presented as the means ± standard deviations. Differences were considered to be statistically significant when $p < 0.05$. AUC from time = 0 to the last sampling point was calculated by the linear trapezoidal rule.

Results

***In vitro* transport of fluoroquinolones.** We used the polarized canine kidney cell line MDCK-II and its subclones transduced with murine Bcrp1 and human BCRP cDNAs, to test the possible role of murine Bcrp1 and human BCRP in the *in vitro* transport of ciprofloxacin, ofloxacin and norfloxacin. The parental and transduced cell lines were grown to confluent polarized monolayers on porous membrane filters, and vectorial transport of the fluoroquinolones (10 μM) across the monolayers was determined. In the MDCK-II parental cell line, apically and basolaterally directed translocation were similar (Fig. 1A, 2A and 3A). In the Bcrp1-transduced MDCK-II cell lines, apically directed translocation was highly increased and basolaterally directed translocation drastically decreased for the three compounds (Fig. 1B, 2B and 3B). When the selective Bcrp1 inhibitor Ko143 was used (Allen et al., 2002), the Bcrp1/BCRP mediated transport was completely inhibited (Fig. 1C, 1E, 2C, 2E, 3C and 3E), resulting in a vectorial translocation pattern equal to that of the MDCK-II parental cell line. In the human BCRP-transduced cell line, the difference in the directional transport was lower than in the murine Bcrp1-transduced cell line (Fig. 1D, 2D and 3D). This may be due to an effectively lower level of the human protein in the cell line used, as also suggested by the nearly 5-fold smaller reduction in mitoxantrone accumulation in these cells (see Fig. 4 below). Alternatively, human BCRP may have reduced transport efficiency for all of these substrates. These results thus show efficient transport of ciprofloxacin, ofloxacin and norfloxacin by murine Bcrp1 and moderate transport by human BCRP in the cell lines used.

Mitoxantrone accumulation studies. In order to further characterize the interactions of the fluoroquinolones with Bcrp1/BCRP, the ability of these compounds to reverse the reduced mitoxantrone accumulation in murine Bcrp1- and BCRP-

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

expressing cell lines was tested in flow cytometry experiments (Fig. 4). BCRP inhibition with Ko143 increases the accumulation of mitoxantrone in Bcrp1- and BCRP-transduced cells and thus increases the median of fluorescence (MF) to levels similar to those in the parental cells. The addition of fluoroquinolones (ciprofloxacin, ofloxacin and norfloxacin) did not affect the accumulation of mitoxantrone (5 μM) in Bcrp1- and BCRP-transduced cells, indicating that these compounds were not effective BCRP/Bcrp1 inhibitors at 100-200 μM.

Plasma pharmacokinetics of ciprofloxacin in Bcrp1$^{-/-}$ and wild-type mice. To assess whether the *in vitro* Bcrp1-mediated transport of fluoroquinolones was also relevant *in vivo*, we studied ciprofloxacin pharmacokinetics in Bcrp1$^{-/-}$ and wild-type male mice. We determined the plasma concentration of ciprofloxacin after its oral and intravenous administration (10 mg/kg) in both types of mice (Fig. 5). Plasma concentration was more than 2-fold increased in Bcrp1$^{-/-}$ compared to wild-type mice (1.77 ± 0.73 *versus* 0.85 ± 0.39 μg/ml, $p < 0.01$) 15 min after oral administration (Fig. 5A). In the case of i.v. administration (Fig. 5B), the AUC of the Bcrp1$^{-/-}$ was 1.5-fold higher compared with the wild-type mice (48.63 ± 5.66 *versus* 33.10 ± 4.68 min.μg/ml, $p < 0.05$). Bcrp1 thus appears to be both an important determinant for the oral availability and the elimination of ciprofloxacin.

Secretion of ciprofloxacin into the milk in Bcrp1$^{-/-}$ and wild-type mice. To test whether Bcrp1 plays a role in the secretion of fluoroquinolones into milk, ciprofloxacin (10 mg/kg) was administered i.v. to lactating Bcrp1$^{-/-}$ and wild-type females, and 10 min after administration, milk and blood were collected. The data obtained from the analysis of milk and plasma concentrations are shown in Fig. 6. The concentration of ciprofloxacin was 2-fold lower in the milk of Bcrp1$^{-/-}$ mice (2.19 ± 0.13 *versus* 4.44 ± 0.84 μg/ml, $p < 0.01$). Ciprofloxacin appears to be actively transported into the milk of

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

mice by Bcrp1, since the milk-to-plasma ratio for wild-type mice was 2-fold higher compared to Bcrp1$^{-/-}$ lactating females (3.08 ± 0.92 *versus* 1.59 ± 0.08, $p < 0.05$).

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Discussion

In this study, we show that three widely used fluoroquinolone antibiotics (ciprofloxacin, ofloxacin and norfloxacin) are transported by Bcrp1/BCRP, and that murine Bcrp1 affects the oral availability, pharmacokinetics and milk secretion of ciprofloxacin. As seen with some other transported Bcrp1/BCRP substrates (unpublished data), these fluoroquinolones are not effective inhibitors of Bcrp1/BCRP.

Our results show efficient *in vitro* transport of ciprofloxacin, ofloxacin and norfloxacin by murine Bcrp1 and more moderate transport by human BCRP. These apparent differences in efficiency of transport between the murine and human homologues (Fig., 1, 2 and 3) may be due to a lower effective expression level of the human BCRP construct compared to Bcrp1 in the cell lines used, as the prototype BCRP substrate mitoxantrone was also less affected in the BCRP- compared to the Bcrp1-expressing cell line (Fig. 4). However, we cannot exclude that there are differences in the affinity/selectivity of BCRP and Bcrp1 for substrates, as has been hypothesized by Mizuno et al. (2004).

An additional pathway for secretion of ciprofloxacin in Caco-2 cells, which is distinct from both P-glycoprotein and MRP2, has been suggested (Lowes and Simmons, 2002). BCRP is a good candidate responsible for the ciprofloxacin secretion in Caco-2 cells, since this cell line also expresses functional BCRP (Xia et al., 2005).

Our *in vivo* data suggest that Bcrp1 restricts ciprofloxacin oral bioavailability and contributes to overall ciprofloxacin elimination (Fig. 5). From the present study, we cannot exclude the presence of additional transporters for these fluoroquinolones. Basolateral transport mechanisms for ciprofloxacin in intestine and liver have been reported (Griffith et al., 1994). However, the most obvious apical ABC transporters P-glycoprotein and MRP2 do not appear to transport ciprofloxacin (Lowes and Simmons,

2002; Michot et al., 2004). The fact that other fluoroquinolones, such as grepafloxacin, are transported by MDR1 and MRP2, demonstrates that multiple ABC transporters could contribute to the overall transepithelial secretion of fluoroquinolones (Lowes and Simmons, 2002). In any case, BCRP seems to be the secretory mechanism common to at least the three fluoroquinolones tested in this study.

Xenobiotic transport in the mammary gland can have tremendous clinical, toxicological and nutritional implications. Passive and active transport processes are involved in the transfer of many drugs into breast milk. *In vitro* models of passive drug transfer identify drug protein binding, drug ionization, and fat partitioning as the critical determinants of passive drug transfer (Fleishaker et al., 1987; Begg and Atkinson, 1993). However, recently, an important role of Bcrp1 in the active secretion and concentration of several drugs and carcinogenic xenotoxins into milk has been established (Jonker et al., 2005; Merino et al., 2005a, van Herwaarden et al., 2005). Our data clearly demonstrate that the presence of Bcrp1 in the mammary gland is also an important factor in the transfer of fluoroquinolones into the milk, as indicated by the 2-fold higher milk-to-plasma ratio of wild-type compared to Bcrp1$^{-/-}$ mice (Fig. 6). Extrapolating our data to other species, the ciprofloxacin milk-to-plasma ratio for wild-type mice we measured (3.08 ± 0.92) is close to the value obtained in other species, such as rabbits (3.61) and humans (4.67) (Gardner et al., 1992; Aramayona et al., 1996), and higher than the predicted milk-to-plasma ratio of 1.5 that physicochemical principles would suggest in human milk (Agatanovic-Kustrin et al., 2002). This value is similar to the ciprofloxacin milk-to-plasma ratio for Bcrp1$^{-/-}$ mice (1.59 ± 0.08). All this suggests that our findings may be relevant to other species, including humans.

Milk secretion and pharmacokinetics of another antibiotic, nitrofurantoin, have been recently shown to be highly affected by Bcrp1 (Merino et al., 2005a). The results

so far suggest that this transporter could be a very important factor in the study and evaluation of contamination of milk with antibiotics and residues, and in the safety of antibiotic application during lactation.

The data presented here may already have other important biological and therapeutic significance. The expression and function of BCRP is variable, depending on different factors, such as coadministered drugs or natural products (Sugimoto et al., 2005), gender (Merino et al., 2005b) or genetic polymorphisms (Sparreboom et al., 2004). Fluoroquinolones are barely metabolized (Montay et al., 1994), so their potential to be affected in their pharmacokinetics by coadministered BCRP inhibitors may have significant consequences *in vivo*. The reciprocal situation, inhibition of BCRP by fluoroquinolones, is less probable, since our mitoxantrone accumulation results suggest that fluoroquinolones do not efficiently inhibit Bcrp1/BCRP *in vitro* (Fig. 4). However, we cannot completely exclude this possibility when substrates distinct from mitoxantrone would be used. In any case, all these factors could modify the pharmacokinetics and milk secretion of fluoroquinolones and thus their local toxicity, their effect on the newborn and even their bactericidal activity. Fluoroquinolone antibiotic activity is concentration dependent and suboptimal concentrations due to variations (either inter- or intra-individual) in BCRP activity could be associated with insufficient eradication of microorganism and potentially rapid emergence of resistance (Michot et el., 2004). Finally, elevated concentrations of the fluoroquinolones could increase the incidence of adverse effects not only in adults but also in infants.

References

Allen JD, van Loevezijn A, Lakhai JM, van der Valk M, van Tellingen O, Reid G, Schellens JHM, Koomen G-J and Schinkel AH (2002) Potent and specific inhibition of the breast cancer resistance protein multidrug transporter *in vitro* and in mouse intestine by a novel analogue of fumitremorgin C. *Mol Cancer Ther* 1:417-425.

Agatonovic-Kustrin S, Ling LH, Tham SY and Alany RG (2002) Molecular descriptors that influence the amount of drugs transfer into human breast milk *J Pharm Biomed Anal* 29:103-19

Aramayona JJ, Mora J, Fraile LJ, Garcia MA, Abadia AR and Bregante MA (1996) Penetration of enrofloxacin and ciprofloxacin into breast milk, and pharmacokinetics of the drugs in lactating rabbits and neonatal offspring. *Am J Vet Res* 57:547-553.

Begg EJ and Atkinson HC (1993) Modelling of the passage of drugs into milk. *Pharmacol Ther* 59:301-10.

Brunner H and Zeiler H-J (1988) Oral ciprofloxacin treatment for Salmonella typhimurium infection of normal and immunocompromised mice. *Antimicrob Agents Chemother* 32:57-62.

Burger H, Van Tol H, Boersma AW, Brok M, Wiemer EA, Stoter G and Nooter K (2004) Imatinib mesylate (STI571) is a substrate for the breast cancer resistance protein (BCRP)/ABCG2 drug pump. *Blood* 104:2940-2942.

Cao CX, Silverstein SC, Neu C and Steinberg TH (1992) J774 macrophages secrete antibiotics via organic anion transporters. *J Infect Dis* 165:322-328.

Cavet Me, West M and Simmons NL (1997) Fluoroquinolone (ciprofloxacin) secretion by human intestinal epithelial (Caco-2) cells. *Br J Pharmacol* 121:1567-1578.

Dan M, Weidekamm E, Sagiv R, Portmann R and Zakut H (1993) Penetration of fleroxacin into breast milk and pharmacokinetics in lactating women. A*ntimicrob Agents Chemother* 37:293-296.

Dautrey S, Felice K, Petiet A, Lacour B, Carbon C and Farinotti R (1999) Active intestinal elimination of ciprofloxacin in rats: modulation by different substrates. *Br J Pharmacol* 127:1728-1734.

Fleishaker JC, Desai N, McNamara PJ (1987) Factors affecting the milk-to-plasma drug concentration ratio in lactating women: physical interactions with protein and fat. *J Pharm Sci* 76:189-93.

Gardner DK, Gabbe SG and Harter C (1992) Simultaneous concentrations of ciprofloxacin in breast milk and in serum in mother and breast-fed infant. *Clin Pharm* 11:352-354.

Giamarellou H, Kolokythas E, Petrikkos G, Gazis J, Aravantinos D and Sfitakis P (1989) Pharmacokinetics of three newer quinolones in pregnant and lactating women *Am J Med* 87:49S-51S.

Griffiths NM, Hirst BH and Simmons NL (1993) Active secretion of the fluoroquinolone ciprofloxacin by human intestinal epithelial Caco-2 cell layers. *Br J Pharmacol* 108:575-576.

Griffiths NM, Hirst BH and Simmons NL (1994) Active intestinal secretion of the fluoroquinolone antibacterials ciprofloxacin, norfloxacin and pefloxacin; a common secretory pathway? *J Pharmacol Exp Ther* 269:496-502.

Idowu OR and Peggins JO (2004) Simple, rapid determination of enrofloxacin and ciprofloxacin in bovine milk and plasma by high-performance liquid chromatography with fluorescence detection. *J Pharm Biomed Anal* 35:143-153.

Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JHM and Schinkel AH (2000) Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J Natl Cancer Inst* 92:1651-1656.

Jonker JW, Merino G, Musters S, van Herwaarden AE, Bolscher E, Wagenaar E, Mesman E, Dale TC and Schinkel AH (2005) The Breast Cancer Resistance Protein (BCRP/ABCG2) concentrates drugs and carcinogenic xenotoxins into milk. *Nat Med* 11:127-129.

Lamp KC, Bailey EM and Rybak MJ (1992) Ofloxacin clinical pharmacokinetics. *Clin Pharmacokinet* 22:32-46.

Lowes S and Simmons N (2002) Multiple pathways for fluoroquinolone secretion by human intestinal epithelial (Caco-2) cells. *Br J Pharmacol* 135:1263-75.

Marazuela MD and Moreno-Bondi MC (2004) Multiresidue determination of fluoroquinolones in milk by column liquid chromatography with fluorescence and ultraviolet absorbance detection. *J Chromatogr A* 1034:25-32.

Merino G, Jonker JW, Wagenaar E, van Herwaarden AE, Schinkel AH. (2005a) The Breast Cancer Resistance Protein (BCRP/ABCG2) affects pharmacokinetics, hepatobiliary excretion and milk secretion of the antibiotic nitrofurantoin. *Mol Pharmacol* 67:1758-1764.

Merino G, van Herwaarden AE, Wagenaar E, Jonker JW, Schinkel AH (2005b) Sex-dependent expression and activity of the ABC transporter Breast Cancer Resistance Protein (BCRP/ABCG2) in liver. *Mol Pharmacol* 67:1765-1771.

Michot J-M, van Bambeke F, Mingeot-Leclercq M-P and Tulkens PM (2004) Active efflux of ciprofloxacin from J774 macrophages through an MRP-like transporter. *Antimicrob Agents Chemother* 48:2673-2682

Mizuno N, Suzuki M, Kusuhara H, Suzuki H, Takeuchi K, Niwa T, Jonker JW and Sugiyama Y (2004) Impaired renal excretion of 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-piridylmethyl) benzothiazole (E3040) sulfate in breast cancer resistance protein (Bcrp1/Abcg2) knockout mice. *Drug Metab Dispos* 32:898-901.

Montay G, Vergniol JC, Ebmeier M, Le Roux Y, Guimart C, Frydman A, Chassard D and Thebault JJ (1994) Pharmacokinetics of sparfloxacin in humans after single oral administration at doses of 200, 400, 600, and 800 mg. *Clin Pharmacol* 34(11):1071-1076.

Parry MG, Smego DA and Digiovanni MA (1988) Hepatobiliary kinetics and excretion of ciprofloxacin. *Antimicrob Agents Chemother* 32:982-985.

Pavek P, Merino G, Wagenaar E, Bolscher E, Novotna M, Jonker JW and Schinkel AH (2005) Human breast cancer resistance protein (BCRP/ABCG2): interactions with steroid drugs, hormones, the dietary carcinogen PhIP, and transport of cimetidine. *J Pharmacol Exp Ther* 312:144-152.

Rabbaa L, Dautrey S, Colas-Linhart N, Carbon C and Farinotti R (1996) Intestinal elimination of ofloxacin enantiomers in the rat: evidence of a carrier-mediated process. *Antimicrob Agents Chemother* 36:2506-2511.

Rohwedder RW, Bergan T, Thorsteinsson SB and Scholl H (1990) Transintestinal elimination of ciprofloxacin. *Diagn Microbiol Infect Dis* 13:127-133.

Sörgel F, Jaehde U, Naber KG and Stephan U (1989) Pharmacokinetic disposition of quinolones in human body fluids and tissues. *Clin Pharmacokinet* 16:51S-68S

Sparreboom A, Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, Twelves C, Verweij J and McLeod HL (2004) Diflomotecan pharmacokinetics in relation to ABCG2 421 C>A genotype. *Clin Pharmacol Ther* 76:38-44.

Stahlmann R (2002) Clinical toxicological aspects of fluoroquinolones. *Toxicol Lett* 27:269-77.

Sugimoto Y, Tsukara S, Ishikawa E and Mitsuhashi J (2005) Breast cancer resistance protein: molecular target for anticancer drug resistance and pharmacokinetics/pharmacodynamics. *Cancer Sci* 96:457-465.

van Herwaarden AE, Jonker JW, Wagenaar E, Brinkhuis RF, Schellens JH, Beijnen JH and Schinkel AH (2003) The breast cancer resistance protein (Bcrp1/Abcg2) restricts exposure to the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Cancer Res* 63:6447-6452.

van Herwaarden AE, Wagenaar E, Karnekamp B, Merino G, Jonker JW, Schinkel AH (2005) Breast cancer resistance protein (Bcrp1/Abcg2) reduces systemic exposure of the dietary carcinogens aflatoxin B1, IQ and Trp-P-1 but also mediates their secretion into breast milk. *Carcinogenesis* [Epub ahead of print]

Xia CQ, Liu N, Yang D, Miwa G, Gan LS (2005) Expression, localization, and functional characteristics of breast cancer resistance protein in Caco-2 cells. *Drug Metab Dispos* 33:637-643.

FOOTNOTES

This work was supported by grant AGL2003-03888 to AA and Juan de la Cierva grant to GM from the Ministerio de Ciencia y Tecnología, Spain

Reprint requests: Julio G. Prieto, Department of Physiology, Faculty of Veterinary Medicine, Campus de Vegazana 24071 León, Spain. Telephone: +34-987291263. FAX: +34-987291267. E-mail: dfijpf@unileon.es

LEGENDS FOR FIGURES

Fig. 1. Transepithelial transport of ciprofloxacin (10 μM) in MDCKII (parent) (A), MDCKII-Bcrp1 (B and C) and MDCKII-BCRP (D and E), monolayers. The experiment was started with the addition of ciprofloxacin to one compartment (basolateral or apical). After 2 and 4 hours, the percentage of drug appearing in the opposite compartment was measured by HPLC and plotted. BCRP inhibitor Ko143 (C and E) was present as indicated. Results are the means; error bars (sometimes smaller than the symbols) indicate the standard deviations (n = 3-4, ***, $p < 0.001$). Closed circles: translocation from the basolateral to the apical compartment; open circles: translocation from the apical to the basolateral compartment.

Fig. 2. Transepithelial transport of ofloxacin (10 μM) in MDCKII (parent) (A), MDCKII-Bcrp1 (B and C) and MDCKII-BCRP (D and E), monolayers. The experiment was started with the addition of ofloxacin to one compartment (basolateral or apical). After 2 and 4 hours, the percentage of drug appearing in the opposite compartment was measured by HPLC and plotted. BCRP inhibitor Ko143 (C and E) was present as indicated. Results are the means; error bars (sometimes smaller than the symbols) indicate the standard deviations (n = 3-4, *, $p < 0.05$, **, $p < 0.01$). Closed circles: translocation from the basolateral to the apical compartment; open circles: translocation from the apical to the basolateral compartment.

Fig. 3. Transepithelial transport of norfloxacin (10 μM) in MDCKII (parent) (A), MDCKII-Bcrp1 (B and C) and MDCKII-BCRP (D and E), monolayers. The experiment was started with the addition of norfloxacin to one compartment (basolateral or apical). After 2 and 4 hours, the percentage of drug appearing in the opposite compartment was

DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

measured by HPLC and plotted. BCRP inhibitor Ko143 (C and E) was present as indicated. Results are the means; error bars (sometimes smaller than the symbols) indicate the standard deviations (n = 3-4, *, $p < 0.05$, **, $p < 0.01$). Closed circles: translocation from the basolateral to the apical compartment; open circles: translocation from the apical to the basolateral compartment.

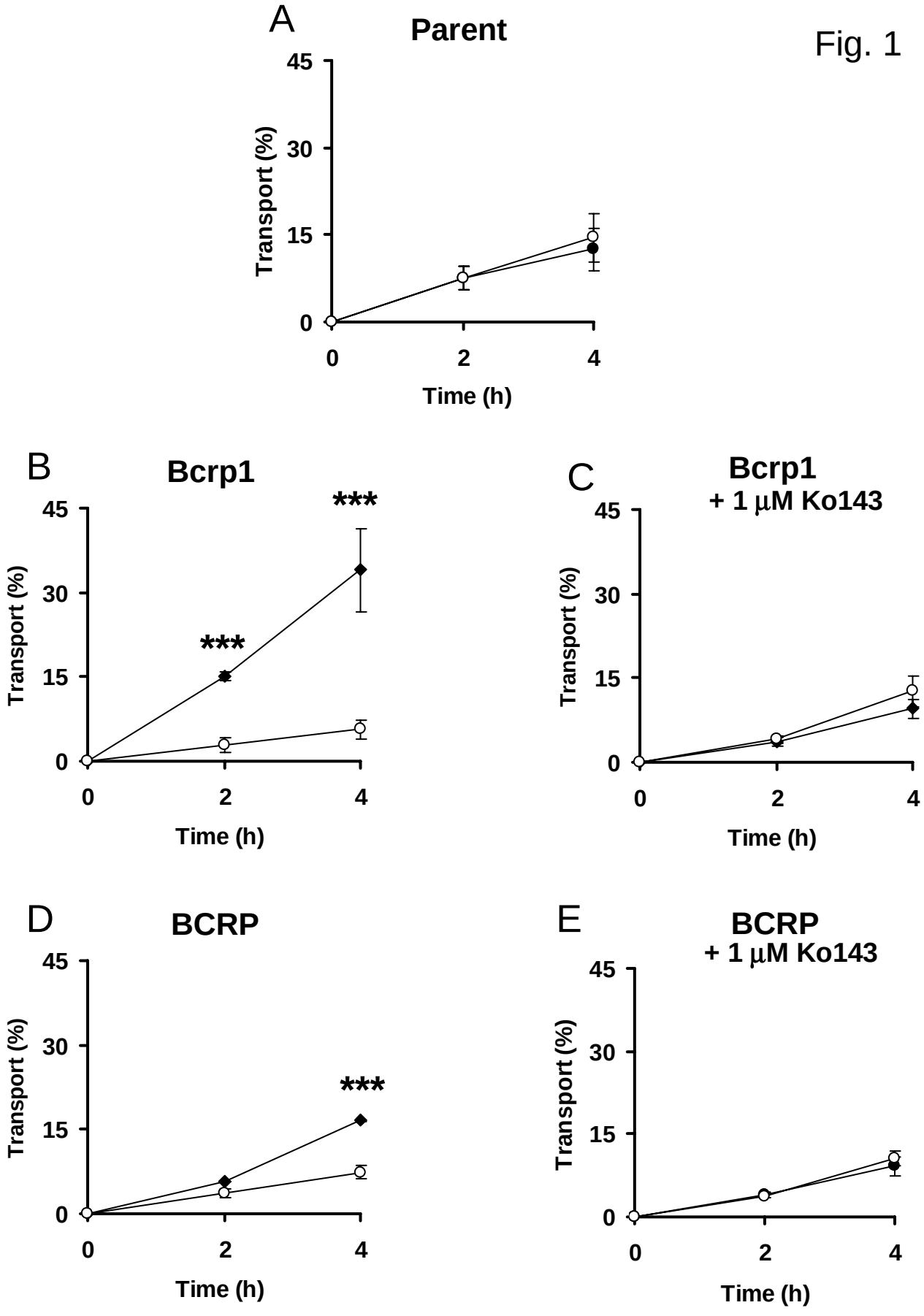
Fig. 4. Effect of the three fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin) on accumulation of mitoxantrone (at 5μM) in parent MDCKII cells and in their BCRP- and Bcrp1- transduced derivatives. Cells were preincubated with or without Ko143 or fluoroquinolones at the indicated concentrations. Results (units of fluoresecence, median) are expressed as means of at least three experiments; error bars indicate the standard deviations.

Fig. 5. Plasma concentrations after oral (A) and i.v. (B) administration of ciprofloxacin (10 mg/kg) to wild-type and Bcrp1$^{-/-}$ male mice. Plasma samples were taken 15 min after administration (A) or at several time points during 30 min (B). Plasma levels of ciprofloxacin were determined by HPLC. Results are the means; error bars indicate the standard deviations (n = 4-9, *, $p < 0.05$, **, $p < 0.01$)

Fig. 6. Plasma and milk concentration (A) and milk-to-plasma ratio (B) of ciprofloxacin in wild-type and Bcrp1$^{-/-}$ lactating females. Ciprofloxacin (10 mg/kg) was administered i.v. to mice and milk and plasma were collected after 10 min and analyzed by HPLC. Results are the means; error bars (sometimes smaller than the symbols) indicate the standard deviations (n = 3, *, $p < 0.05$, **, $p < 0.01$).

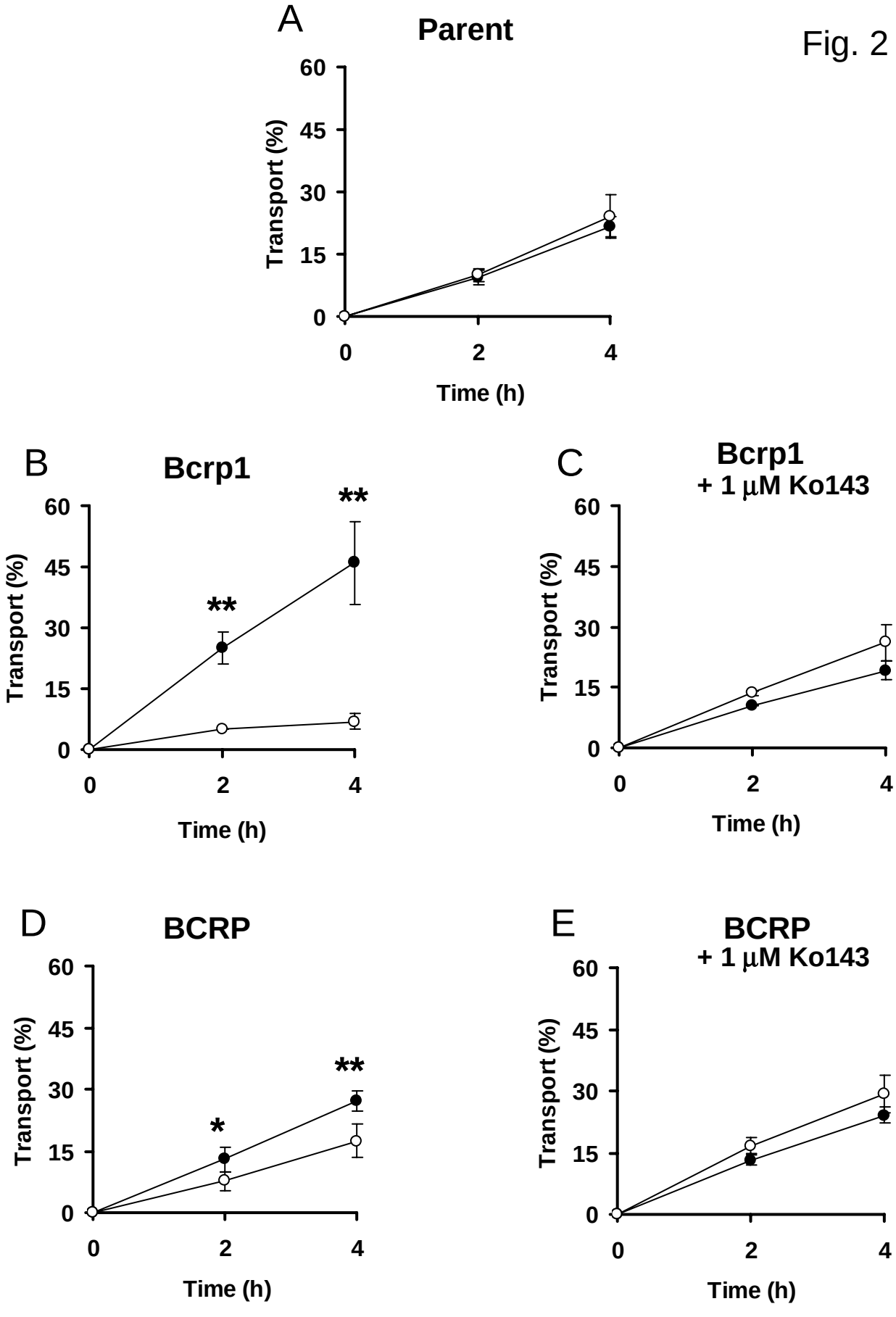
DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 1



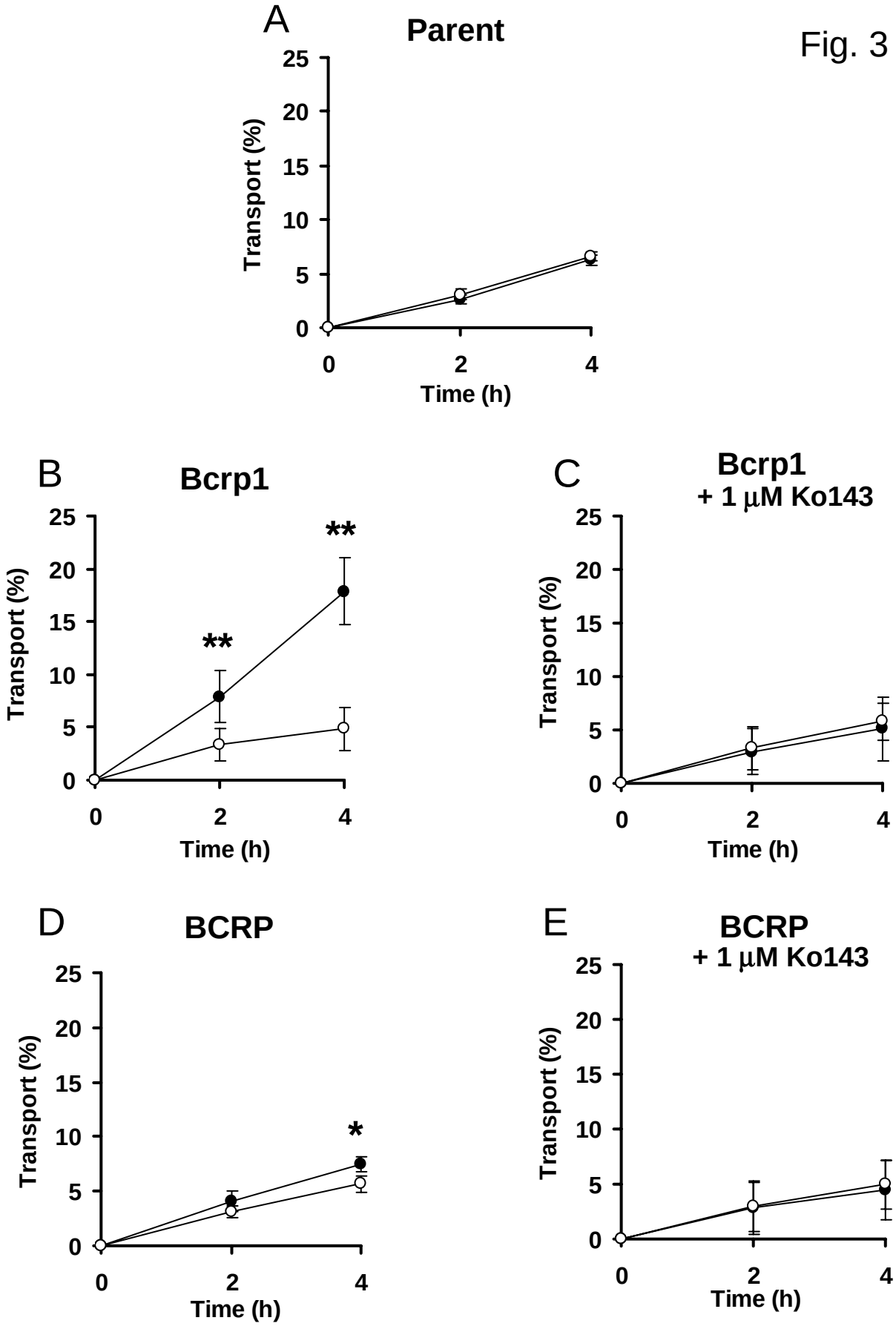
DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 2



DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 3



DMD Fast Forward. Published on January 24, 2006 as DOI: 10.1124/dmd.105.008219
This article has not been copyedited and formatted. The final version may differ from this version.

Fig. 4

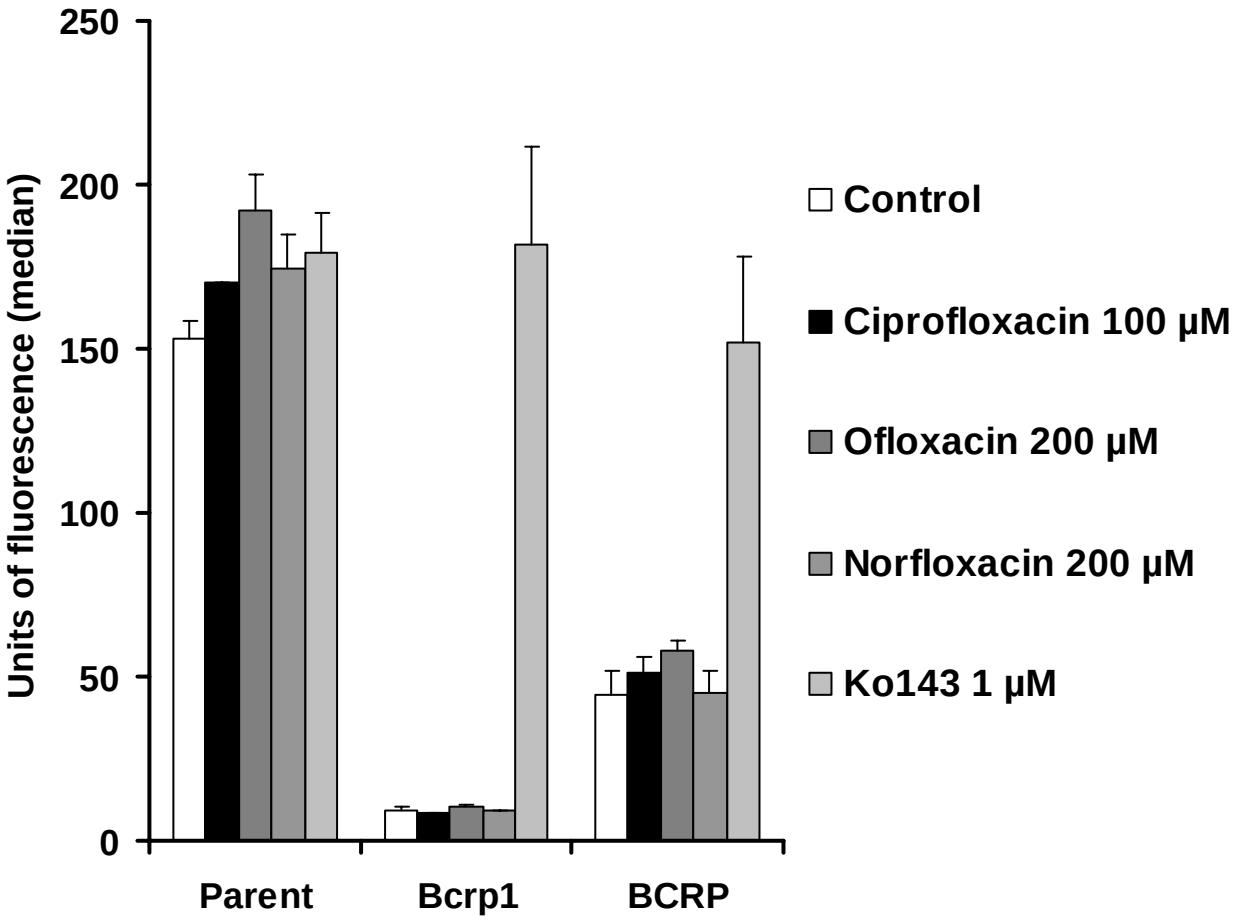


Fig. 5

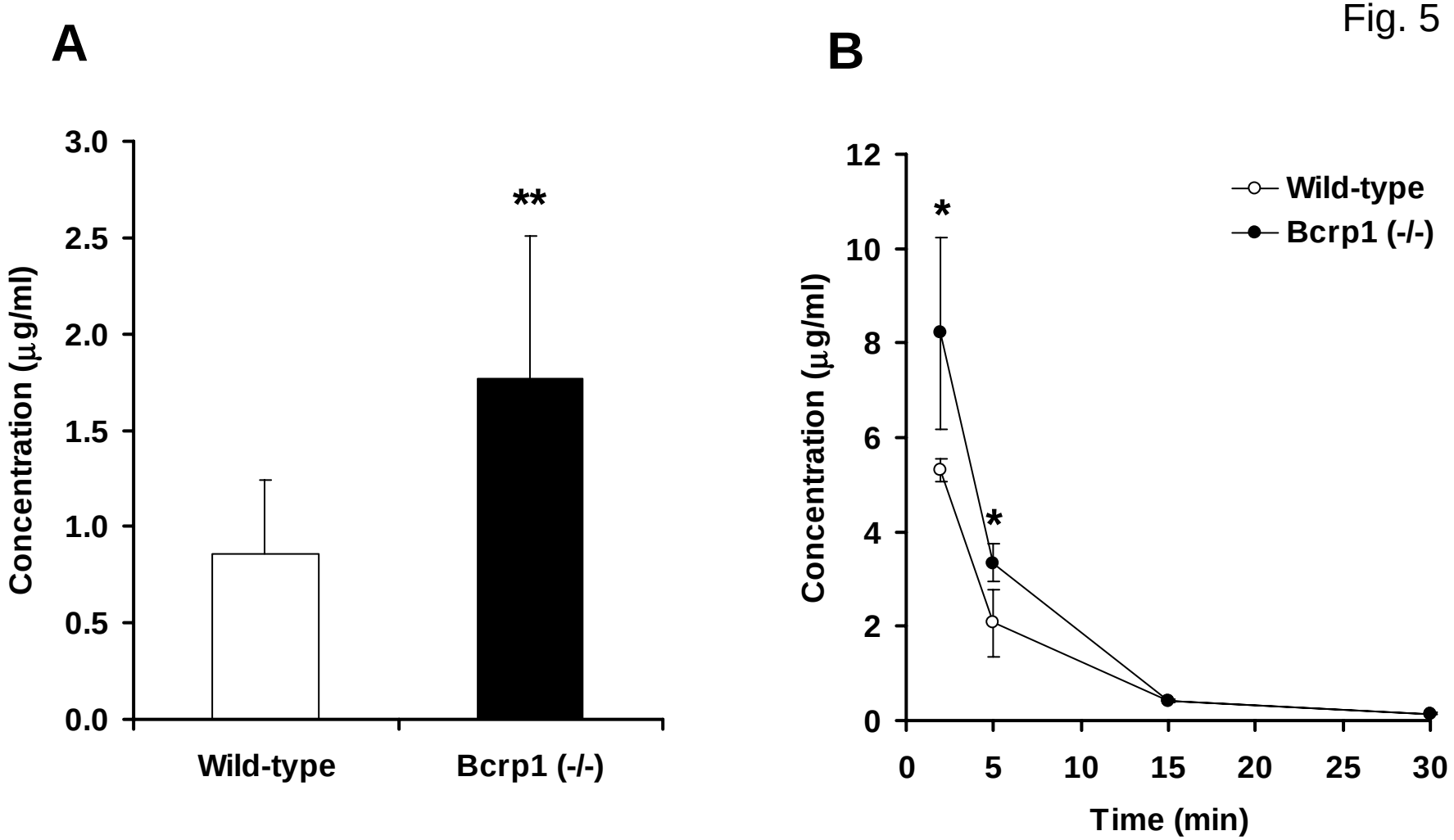


Fig. 6