molecular pharmaceutics

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Breast Cancer Resistance Protein (Bcrp/abcg2) Is a Major Determinant of Sulfasalazine Absorption and Elimination in the Mouse

Hani Zaher,* Anis A. Khan, Joe Palandra, Timothy G. Brayman, Linning Yu, and Joseph A. Ware

Department of Pharmacokinetics, Dynamics, and Metabolism, Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Road, Ann Arbor, Michigan 48105

Received December 27, 2005

Abstract: Sulfasalazine is used in the treatment of ulcerative colitis, Crohn's disease, and rheumatoid arthritis. When administered orally, sulfasalazine is poorly absorbed with an estimated bioavailability of 3−12%. Recent studies using the T-cell line (CEM) have shown that sulfasalazine is a substrate for the ATP-binding cassette (ABC) efflux pump ABCG2. ABCG2 is known to efflux a number of xenobiotics and appears to be a key determinant of efficacy and toxicity of ABCG2 substrates. To date, there has not been any systematic study on the mechanisms involved in the transport of sulfasalazine in vivo. Accordingly, we investigated whether Bcrp (abcg2) is involved in the disposition of sulfasalazine. After oral administration of 20 mg/kg sulfasalazine, the area under the plasma concentration (AUC) time profile in Bcrp1 $(abcg2)^{-/-}$ knockout (KO) mice was approximately 111-fold higher than that in FVB wild-type (WT) mice. After intravenous administration of 5 mg/kg sulfasalazine, the AUC in Bcrp1 $(abcg2)^{-/-}$ KO mice was approximately 13-fold higher than that in WT mice. Moreover, treatment of WT mice with a single oral dose of gefitinib (Iressa; 50 mg/kg), a known inhibitor of Bcrp, given 2 h prior to administering a single oral dose of sulfasalazine (20 mg/kg), resulted in a 13-fold increase in the AUC of sulfasalazine compared to the AUC in vehicle-treated mice. Since gefitinib is also an inhibitor of P-glycoprotein (P-gp), the impact of P-gp on sulfasalazine absorption in vivo was also examined. The sulfasalazine AUC in $mdr1a^{-/-}$ KO versus WT mice did not differ significantly after either an oral (20 mg/kg) or an intravenous dose (5 mg/kg). We conclude that Bcrp (abcg2) is an important determinant for the oral bioavailability and the elimination of sulfasalazine in the mouse, and that sulfasalazine has the potential to be utilized as a specific in vivo probe of Bcrp (abcg2).

Keywords: BCRP; ABCG2; sulfasalazine; drug absorption; bioavailability; knockout mouse; sulfapyridine

Introduction

Sulfasalazine (5-([*p*-(2-pyridysulfamoyl)phenyl]azo)salicylic acid) is an antiinflammatory agent widely used in humans to treat inflammatory bowel diseases, such as ulcerative colitis and Crohn's disease.[1,2] The drug is also used in the treatment of patients with rheumatoid arthritis.[3,4]

* Corresponding author. Mailing address: Department of Pharmacokinetics, Dynamics, and Metabolism, Pfizer Global Research and Development, Michigan Laboratories, 2800 Plymouth Rd., Ann Arbor, MI 48105. Phone: (734) 622-5057. Fax: (734) 622-4349. E-mail: hani.zaher@pfizer.com.

(1) Peppercorn, M. A. Sulfasalazine. Pharmacology, clinical use, toxicity, and related new drug development. *Ann. Intern. Med.* **1984**, *101*, 377−86.

(2) Klotz, U. Clinical efficacy of oral 5-aminosalicylic acid in the treatment of inflammatory bowel disease. *Am. J. Gastroenterol.* **1985**, *80*, 660.

10.1021/mp050113v CCC: $33.50
Published on Web 02/06/2006

Sulfasalazine is metabolized by bacteria in the lower intestinal tract to sulfapyridine and 5′-aminosalicylic acid.[5,6] Of the two major metabolites, sulfapyridine is relatively well absorbed from the intestine and further metabolized by N-acetylation, while 5′-aminosalicylic acid is not as well absorbed.[5,6] In humans, the absolute bioavailability of orally administered sulfasalazine is less than 15%.[7−9] This lower bioavailability of sulfasalazine in humans has been attributed largely to the limited solubility and poor permeability of this compound.[10]

Using the Caco-2 cell line, it has been shown that sulfasalazine is a substrate for various cellular ATP-binding cassette (ABC) efflux pumps, which may, at least partially, explain its low intestinal absorption in vivo.[11,12] Breast cancer resistance protein (BCRP/ABCG2) is a member of the ABC superfamily of transporters[13,14] that affects the pharmacological and toxicological behavior of many drugs and toxins. This 655-amino acid, half-transporter, is known to actively efflux a range of anticancer drugs, dietary compounds, and food carcinogens such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP).[15−18] ABCG2 is localized in the apical membranes of intestinal enterocytes, colonocytes, placental epithelia, hepatic biliary canalicular membrane, and mammary gland and at the blood−brain barrier. A growing body of literature suggests that ABCG2 is involved with many processes of xenobiotic disposition. In particular, ABCG2 limits the oral bioavailability of topotecan,[17] irinotecan,[18] NMDA antagonists,[20] and nitrofurantoin.[21] Moreover, fetal (ABC-placental) exposure and hematopoietic[22] and brain distribution and permeation can also be impacted by ABCG2.[23] For xenobiotic and drug elimination, ABCG2 contributes to hepatobiliary clearance and both mammary gland epithelia and intestinal secretion of many substrates.[15−21]

Interestingly, a human T-cell line (CEM) selectively

(3) Astbury, C.;Taggart, A. J.; Juby, L.; Zebouni, L.; Bird, H. A. Comparison of the single dose pharmacokinetics of sulfasalazine in rheumatoid arthritis and inflammatory bowel disease. *Ann. Rheum. Dis*. **1990**, *49*, 587−90.

(4) Plosker, G. L.; Croom, K. F. Sulfasalazine: a review of its use in the management of rheumatoid arthritis. *Drugs* **2005**, *65*, 1825−49.

(5) Klotz, U.; Schwab, M. Topical delivery of therapeutic agents in the treatment of inflammatory bowel disease. *Adv. Drug Delivery Rev*. **2005**, *57*, 267−79.

(6) Azadkhan, A. K.; Truelove, S. C.; Aronson, J. K. The disposition and metabolism of sulphasalazine (salicylazosulphapyridine) in man. *Br. J. Clin. Pharmacol*. **1982**, *13*, 523−8.

(7) van Hees, P. A.; Bakker, J. H.; van Tongeren, J. H. Effect of sulphapyridine, 5-aminosalicylic acid, and placebo in patients with idiopathic proctitis: a study to determine the active therapeutic moiety of sulfasalazine. *Gut* **1980**, *21*, 632−5.

(8) Klotz, U. Clinical pharmacokinetic of sulfasalazine, its metabolites and other prodrugs of oral 5-aminosalicylic. *Clin. Pharmacokinet.* **1985**, *10*, 285−302.

(9) Friedman, G. Sulfasalazine and new analogues. *Am. J. Gastroenterol.* **1986**, *81*, 141−4.

(10) Yazdanian, M.; Glynn, S. L.; Wright, J. L.; Hawi, A. Correlating partitioning and Caco-2 cell permeability of structurally diverse small molecular weight compounds. *Pharm. Res*. **1998**, *15*, 1490−4.

(11) Liang, E.; Proudfoot, J.; Yazdanian, M. Mechanisms of transport and structure-permeability relationship of sulfasalazine and its analogs in Caco-2 cell monolayers. *Pharm Res*. **2000**, *17*, 1168−74.

(12) Mols, R.; Deferme, S.; Augustijns, P. Sulfasalazine transport in in-vitro, ex-vivo and in-vivo absorption models: contribution of efflux carriers and their modulation by co-administration of synthetic nature-identical fruit extracts. *J. Pharm. Pharmacol*. **2005**, *57*, 1565−73.

(13) Doyle, L. A.; Yang, W.; Abruzzo, L. V.; Krogmann, T.; Gao, Y.; Rishi, A. K.; Ross, D. D. A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 15665−70.

(14) Allen, J. D.; van Loevezijn, A.; Lakhai, J. M.; van der Valk, M.; van Tellingen, O.; Reid, G.; Schellens, J. H. M.; Koomen, G. J.; Schinkel, A. H. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine. *Mol. Cancer Ther*. **2002**, *6*, 417−25.

(15) Jonker, J. W.; Buitelaar, M.; Wagenaar, E.; Van Der Valk, M. A.; Scheffer, G. L.; Scheper, R. J.; Plosch, T.; Kuipers, F.; Elferink, R. P.; Rosing, H. The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *26*, 15649−54.

(16) van Herwaarden, A. E.; Jonker, J. W.; Wagenaar, E.; Brinkhuis, R. F.; Schellens, J. H.; Beijnen, J. H.; and Schinkel, A. H. 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.* **2003**, *63*, 6447−52.

(17) Jonker, J. W.; Smit, J. W.; Brinkhuis, R. F.; Maliepaard, M.; Beijnen, J. H.; Schellens, J. H. M.; Schinkel, A. H. Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J. Natl. Cancer Inst.* **2000**, *92*, 1651−6.

(18) Stewart, C. F.; Leggas, M.; Schuetz, J. D.; Panetta, J. C.; Cheshire, P. J.; Peterson, J.; Daw, N.; Jenkins, J. J.; Gilbertson, R.; Germain, G. S.; Harwood, F. C.; Houghton, P. J. Gefitinib enhances the antitumor activity and oral bioavailability of irinotecan in mice. *Cancer Res*. **2004**, *64*, 7491−9.

(19) Schellens, J. H.; Maliepaard, M.; Scheper, R. J.; Scheffer, G. L.; Jonker, J. W.; Smit, J. W.; Beijnen, J. H.; Schinkel, A. H. Transport of topoisomerase I inhibitors by the breast cancer resistance protein. Potential clinical implications. *Ann. N.Y. Acad. Sci*. **2000**, *922*, 188−94.

(20) Polli, J. W.; Baughman, T. M.; Humphreys, J. E.; Jordan, K. H.; Mote, A. L.; Webster, L. O.; Barnaby, R. J.; Vitulli, G.; Bertolotti, L.; Read, K. D.; Serabjit-Singh, C. J. The systemic exposure of an N-methyl-D-aspartate receptor antagonist is limited in mice by the P-glycoprotein and breast cancer resistance protein efflux transporters. *Drug Metab. Dispos*. **2004**, *32*, 722−6.

(21) Merino, G.; Jonker, J. W.; Wagenaar, E.; van Herwaarden, A. E.; Schinkel, A. H. The breast cancer resistance protein (BCRP/ABCG2) affects pharmacokinetics, hepatobiliary excretion, and milk secretion of the antibiotic nitrofurantoin. *Mol. Pharmacol*. **2005**, *67*, 1758−64.

(22) Zhou, S.; Zong, Y.; Ney, P. A.; Nair, G.; Stewart, C. F.; Sorrentino, B. P. Increased expression of the Abcg2 transporter during erythroid maturation plays a role in decreasing cellular protoporphyrin IX levels. *Blood* **2005**, *105*, 2571−6.

(23) Cisternino, S.; Mercier, C.; Bourasset, F.; Roux, F.; Scherrmann, J. M. Expression, upregulation and transport activity of the multidrug-resistance protein Abcg2 at the mouse blood-brain barrier. *Cancer Res*. **2004**, *64*, 3296−301.

exposed to increasing concentrations of sulfasalazine resulted in a classical multidrug resistance (MDR) phenotype, in part due to the overexpression of BCRP/ABCG2.[24] Moreover, treatment with the BCRP-specific inhibitor Ko-143 reversed BCRP/ABCG2-mediated resistance in CEM cells.[24] The latter study with CEM cells prompted us to explore whether the functional expression of the ATP-binding cassette (ABC) efflux pump BCRP/ABCG2 could play an important role in sulfasalazine oral absorption in vivo and thereby aid our understanding in the relative contribution of this transporter to the pharmacokinetics of sulfasalazine and possibly therapeutic efficacy.

In this study, using Bcrp1 (abcg2) KO mice, we demonstrate that sulfasalazine is transported by Bcrp (abcg2) and that this transporter is a critical determinant of sulfasalazine disposition and oral bioavailability.

Materials and Methods

Animals. Mice were housed and handled according to Pfizer Global Research guidelines complying with the U.S. Public Health Service Policy for the Care and Use of Laboratory Animals. Bcrp1$^{-/-}$ (FVB.129S6-*Abcg2*^{tm1Ahs}) and WT (FVB) were obtained from Taconic Laboratories (Germantown, NY).[15] Male outbred mdr1aPGP (mdr1a$^{-/-}$ KO) and Crl:CF-1 WT mice were purchased from Charles River Laboratories (Wilmington, MA).[25] All mice used in this study were male and between 9 and 14 weeks of age. Animals were kept in a temperature-controlled environment with a 12-h light/dark cycle and received a standard diet and water ad libitum.

Chemicals. Sulfasalazine, oxasolazine, sulfapyridine, and gefitinib were obtained commercially or from Pfizer Global Research and Development (Kalamazoo, MI). PGE-200 and methylcellulose powder were purchased from Sigma (St. Louis, MO). Isoflurane (IsoFlo) was obtained from Abbott laboratories (Abbott Park, IL). All other compounds used were reagents grade.

Pharmacokinetic Experiments with Bcrp1 (abcg2)$^{-/-}$, mdr1a$^{-/-}$, and WT Mice. For oral administration, sulfasalazine in 0.5% (methylcellulose in sterile phosphate buffer saline) was administered orally by gavage to groups of mice at a dose of 20 mg/kg of body weight. In the intravenous (iv) dose study, sulfasalazine was administered via the tail vein at a dose of 5 mg/kg as previously described by others.[26] At predetermined time points, mice were anesthesized with isoflurane, and blood samples were obtained by cardiac puncture and transferred to heparinized tubes. The samples were centrifuged immediately at 3000*g* for 15 min, and plasma was collected and stored at −80 °C until the time of LC−MS/MS analysis.

Effect of Gefitinib on Bcrp (abcg2)-Mediated Transport of Sulfasalazine. The effect of gefitinib on Bcrp1-mediated transport of sulfasalazine was examined in FVB WT mice. Briefly described, gefitinib was suspended in 5% PGE-200 and 95% methylcellulose to provide a final concentration of 5 mg/mL. Gefitinib was given as a single oral dose (50 mg/kg) 2 h prior to administering a single oral dose of sulfasalazine (20 mg/kg) to mice. For all oral studies, blood samples were collected at predose and 0.25, 0.5, 1, 2, 4, and 24 h after administration. All blood samples were handled as previously described. Sulfasalazine plasma concentrations were determined by LC−MS/MS analyses as described below.

LC−MS/MS Methodology. LC−MS/MS analysis was carried out using a high-performance liquid chromatography system consisting of a Shimadzu binary pump with CTC PAL autosampler interfaced to an API 4000 SCIEX triple-quadrupole tandem mass spectrometer (Applied Biosystems, Foster City, CA). Sulfasalazine, sulfapyridine, and the internal standard (oxasolazine) were separated on a Hypersil Gold column (2.1 × 50 mm, Thermo Electron, 25005-052130). The mobile phase consisted of solvent A (5 mM ammonium formate in water) and solvent B (acetonitrile). The gradient was as follows: solvent B was held at 10% for 0.3 min, linearly ramped from 10% to 75% in 1.7 min, ramped from 75% to 95% in 0.7 min, and then immediately brought back down to 10% for reequilibration. Total run time was 3 min with a flow rate of 0.30 mL/min. The mass spectrometer was operated in negative ion ESI for the detection of sulfasalazine, sulfapyridine, and oxasolazine. Multiple reaction monitoring analysis was performed with the transitions *m*/*z* 397.0→197.0 for sulfasalazine, *m*/*z* 301.0→283.0 for oxasolazine, *m*/*z* 248→184 for sulfapyridine. All raw data was processed using Analyst Software, version 1.4 (Applied Biosystems/MDS Sciex Inc., Ontario, Canada).

The methodology for the extraction of the samples was based on the use of an organic solvent, such as acetonitrile, for protein precipitation. Briefly, plasma samples were thawed and vortexed, 25-μL aliquots were transferred to 96-well plates, 100 μL of acetonitrile was added to wells, and plates were centrifuged at 14000*g* for 10 min. Using a Quadra 96 robotic system (model 320, Tomtec) 100 μL of supernatants from wells was transferred to new 96-well plates and 100 μL of internal standard (100 ng/mL) added to all the samples. Standard samples in the appropriate drug-free matrix

(24) van der Heijden, J.; de Jong, M. C.; Dijkmans, B. A.; Lems, W. F.; Oerlemans, R.; Kathmann, I.; Schalkwijk, C. G.; Scheffer, G. L.; Scheper, R. J.; Jansen, G. Development of sulfasalazine resistance in human T cells induces expression of the multidrug resistance transporter ABCG2 (BCRP) and augmented production of TNFα. *Ann. Rheum. Dis*. **2004**, *63*, 138−43.

(25) Umbenhauer, D. R.; Lankas, G. R.; Pippert, T. R.; Wise, L. D.; Cartwright, M. E.; Hall, S. J.; Beare. C. M. Identification of a P-glycoprotein-deficient subpopulation in the CF-1 mouse strain using a restriction fragment length polymorphism. *Toxicol. Appl. Pharmacol*. **1997**, *146*, 88−94.

(26) Zheng, W.; Winter, S. M.; Mayersohn, M.; Bishop, J. B.; Sipes, I. G. Toxicokinetics of sulfasalazine (salicylazosulfapyridine) and its metabolites in B6C3F1 mice. *Drug Metab. Dispos*. **1993**, *21*, 1091−7.

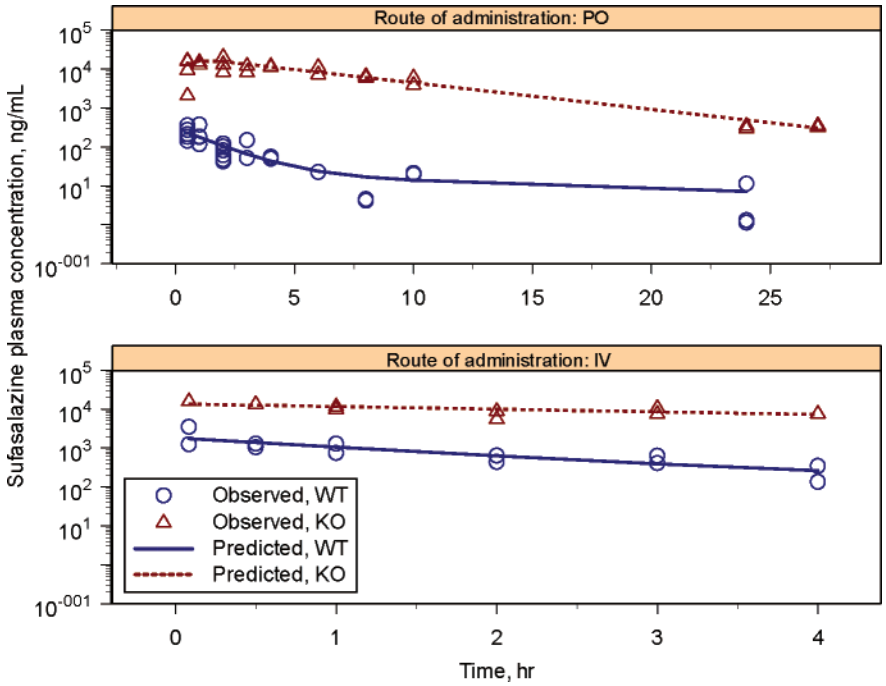

Figure 1. Plasma concentration versus time curves after oral and intravenous administration of sulfasalazine (20 mg/kg, po; 5 mg/kg, iv) to Bcrp1 (abcg2)$^{-/-}$ KO and WT mice. Plasma samples were obtained for 24 h (po; upper graph) or 4 h (iv; lower graph). Plotted are individual data points.

were prepared yielding a concentration range from 8 to 20 000 ng/mL. The limit of detection of sulfasalazine was 2 ng/mL.

Pharmacokinetic Calculations and Statistical Analysis. The concentration−time profiles of sulfasalazine in plasma following oral or intravenous dosing were simultaneously modeled using a nonlinear mixed-effect modeling software, NONMEM version 5.1 (University of California San Francisco, CA). The pharmacokinetic profile of sulfasalazine in WT mice was best described by a two-compartment disposition model. However, in abcg2 KO mice, the data were best described by a one-compartment model. Absolute bioavailability was estimated in both WT and KO mice within the model. AUC from time zero to the last sampling time was calculated by the linear trapezoidal rule using the model-predicted concentrations. Relative exposures were estimated as the ratio of AUC in KO versus WT mice for each dose level and route of administration. Confidence intervals on model predictions were estimated via parametric bootstrapping from the parameter covariance matrix. Five thousand bootstrap samples of model parameters were generated assuming a multivariate normal distribution, and predicted values were calculated for each set of model parameters. The 5th and 95th percentiles were determined from the 5000 predicted values giving a 90% prediction interval (on the mean).

Results

Plasma Pharmacokinetics of Sulfasalazine in Bcrp1 (abcg2)$^{-/-}$ KO and WT Mice. To assess whether Bcrp (abcg2)-mediated transport of sulfasalazine was relevant in vivo, we determined the plasma concentration of sulfasalazine as a function of time, after oral and intravenous administration of sulfasalazine in both WT and KO mice (Figure 1). The absolute and relative bioavailability of sulfasalazine in Bcrp1 (abcg2)$^{-/-}$ and WT mice were also estimated (Tables 1 and 2). Sulfasalazine was cleared from the plasma with an elimination $T_{1/2}$ of 4.4 h in Bcrp1 (abcg2)$^{-/-}$ KO mice and 2.2 h in WT mice. Bcrp1 (abcg2)$^{-/-}$ KO mice had a much lower systemic clearance (CL_s) in comparison with WT mice (Table 1). After oral administration of 20 mg/kg sulfasalazine, the AUC in the Bcrp1 (abcg2)$^{-/-}$ KO mice was spectacularly increased by 111-fold compared with the WT mice (131 822 versus 1189 ng·h/mL). For iv administration (5 mg/kg), the AUC of the Bcrp1 (abcg2)$^{-/-}$ KO was almost 13-fold higher compared with the WT mice, 40 343 versus 3015 ng·h/mL, respectively (Figure 1). The calculated absolute oral availability was significantly increased in Bcrp1 (abcg2)$^{-/-}$ KO compared with WT (37% versus 4%). After an iv injection, C_{max} at time zero, as extrapolated from the model, was approximately 8-fold higher in Bcrp1 (abcg2)$^{-/-}$ KO compared to that in WT, 13 570 versus 1827 ng/mL, respectively (Table 2). Following a single oral dose of sulfasalazine, T_{max} values were achieved within 30 min of dosing for WT versus 120 min for Bcrp1 (abcg2)$^{-/-}$ KO mice. C_{max} in Bcrp1 (abcg2)$^{-/-}$ KO was approximately 70-fold higher than C_{max} in WT (Table 2). After oral administration, sulfapyridine was identified in plasma of Bcrp1 (abcg2)$^{-/-}$ KO and WT mice. Sulfapyridine was present in much higher concentrations (highest C_{max} and AUC) in WT mice than in Bcrp1 (abcg2)$^{-/-}$ KO mice (data not shown). Bcrp (abcg2) thus

Table 1. Pharmacokinetic Parameters of Sulfasalazine in Bcrp1 (abcg2) and mdr1a (WT and KO) Mice[a]

mice	parameters	CL_s ($L \cdot h^{-1} \cdot kg^{-1}$)	V_c ($L \cdot kg^{-1}$)	absolute bioavailability (% *F*)	$T_{1/2}$ (h)
FVB (WT)	mean ± SEM	0.87 ± 0.18	2.74 ± 0.49	4.0 ± 0.6	2.2
	(90% CI)	(0.58−1.15)	(1.93−3.55)	(3−5)	
Bcrp1 KO	mean ± SEM	0.06 ± 0.004	0.37 ± 0.02	36.6 ± 3.2	4.4
	(90% CI)	(0.05−0.06)	(0.33−0.41)	(31−42)	
Crl:CF-1 (WT)	mean ± SEM	0.54 ± 0.16	1.82 ± 0.51	3.9 ± 1.3	2.3
	(90% CI)	(0.28−0.79)	(0.99−2.65)	(2−6)	
mdr1a KO	mean ± SEM	0.47 ± 0.09	2.21 ± 0.42	6.4 ± 1.2	3.3
	(90% CI)	(0.33−0.61)	(1.52−2.86)	(5−8)	

[a] Pharmacokinetic parameters are reported as means ± SEM with 90% confidence intervals.

Table 2. Sulfasalazine C_{max} and Exposure (AUC) in Bcrp1 (abcg2) and mdr1a (WT and KO) Mice Following Intravenous and Oral Administration

mice	route	dose ($mg \cdot kg^{-1}$)	C_{max} (ng/mL^{-1})[a]		AUC ($ng \cdot h \cdot mL^{-1}$)			relative exposure, AUC_{KO}/AUC_{WT}
			WT	KO	duration (h)	WT	KO	
Bcrp1	iv	5	1827	13570	0−4	3015	40343	13
	po	20	233	16176	0−24	1189	131822	111
Mdr1a	iv	5	2749	2266	0−6	5131	3504	1
	po	20	349	440	0−24	1098	1781	2

[a] Intravenous = C_{max} at time zero was extrapolated from the model; oral = visual C_{max} from raw data.

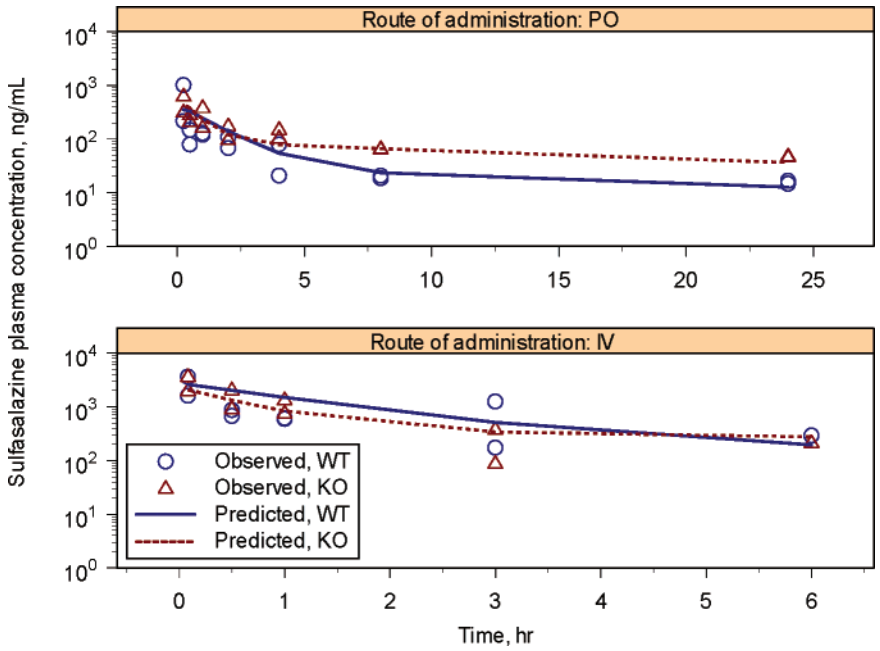

Figure 2. Plasma concentration versus time curves after oral and intravenous administration of sulfasalazine (20 mg/kg, po; 5 mg/kg, iv) to mdr1a$^{-/-}$ KO and WT mice. Plasma samples were taken during 24 h (po; upper graph) or 6 h (iv; lower graph). Plotted are individual data points.

appears to be an important determinant for both the oral availability and the elimination of sulfasalazine in the mouse.

Plasma Pharmacokinetics of Sulfasalazine in mdr1a$^{-/-}$ KO and WT Mice. The plasma concentration−time profiles of sulfasalazine in mdr1a$^{-/-}$ KO and WT were quite similar regardless of the route of administration. After oral administration of 20 mg/kg sulfasalazine, the AUC in mdr1a$^{-/-}$ KO mice was slightly higher than that in the WT mice, 1781 versus 1098 ng·h/mL, respectively (Figure 2 and Table 2). Following iv administration (5 mg/kg), the sulfasalazine AUC in the mdr1a$^{-/-}$ KO was slightly lower than that in WT mice, 3504 versus 5131 ng·h/mL, respectively (Figure 2 and Table 2). The extrapolated iv C_{max} at time zero was very similar between both genotypes (Table 2). Sulfasalazine

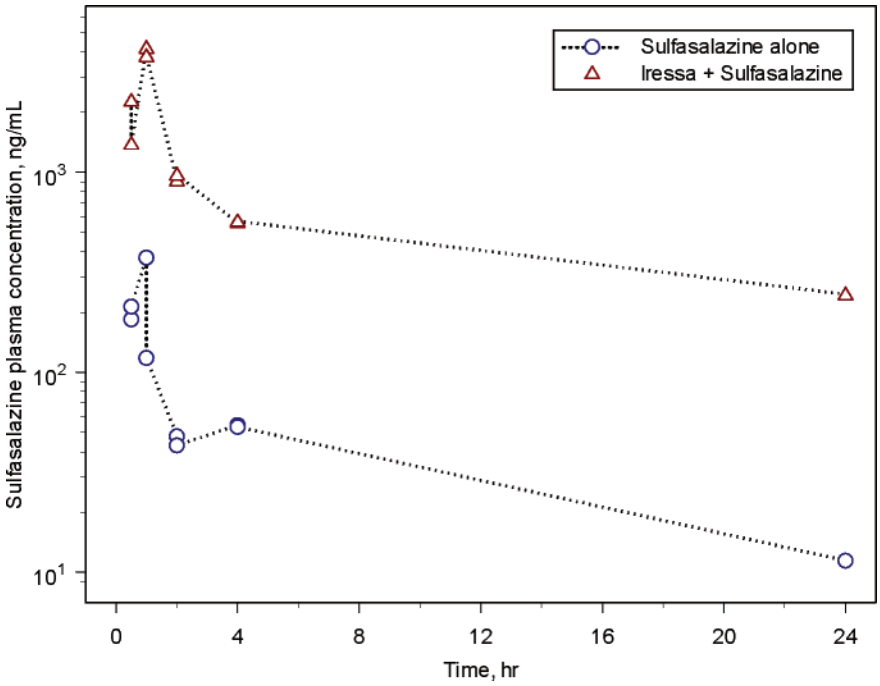

Figure 3. Plasma concentration versus time curve after oral administration of sulfasalazine (20 mg/kg) alone or combined with gefitinib (50 mg/kg, Iressa) gavage 2 h prior to sulfasalazine administration. Plotted are individual data points.

CL_s and $T_{1/2}$ were very comparable in both genotypes (Table 1). These data suggest that P-gp may play only a minor role in sulfasalazine intestinal absorption or pharmacokinetics.

Effect of Gefitinib on Bcrp (abcg2)-Mediated Transport of Sulfasalazine. The epidermal growth factor receptor (EGFR) inhibitor gefitinib (ZD1839, Iressa) has recently been reported to inhibit human ABCG2 in vitro.[27] To further demonstrate that Bcrp (abcg2) plays a major role in sulfasalazine-mediated absorption, male WT mice were pretreated with a single oral gavage of gefitinib (50 mg/kg) 2 h prior to a single oral dose of sulfasalazine (20 mg/kg). The plasma concentration of sulfasalazine as a function of time, in treated and nontreated WT mice, is shown in Figure 3. The AUC of the gefitinib-treated mice increased approximately 13-fold compared to that of vehicle treated WT mice (14 002 versus 1057 ng·h/mL). This chemical inhibition study supports a strong role for abcg2 efflux with respect to sulfasalazine absorption in mice.

Discussion

Sulfasalazine remains the mainstay of treatment of inflammatory bowel diseases such as ulcerative colitis.[1,2] While the target organ of this disease is the large intestine, the reason for sulfasalazine's poor bioavailability was previously thought to be related to less than optimal solubility and permeability.[9,10] Recent studies by der Heijden and colleagues have shown that sulfasalazine could be a BCRP (ABCG2) substrate in vitro.[24] We now show that Bcrp (Abcg2) has a critical role in sulfasalazine absorption in vivo. Our data clearly show that sulfasalazine is an excellent substrate of Bcrp (abcg2) and that murine abcg2 restricts the oral absorption of sulfasalazine. In fact, the contribution of Bcrp (abcg2) to the bioavailability of sulfasalzine is quite remarkable. The absolute F for sulfasalazine varied between 4% and 37% in WT and Bcrp $(abcg2)^{-/-}$ KO mice, respectively. Our data clearly show that Bcrp (abcg2) plays a major role in the intestinal absorption of sulfasalazine as demonstrated by the large difference in the AUC between WT and Bcrp $(abcg2)^{-/-}$ KO mice (111-fold higher in the Bcrp $(abcg2)^{-/-}$ KO mice compared to WT). Our data suggest the intestinal Bcrp (abcg2) restricts sulfasalazine oral bioavailability by reducing its intestinal absorption (Figure 1 and Table 1). Interestingly, systemic clearance (CL_s) of sulfasalazine in Bcrp1 $(abcg2)^{-/-}$ KO mice was also dramatically decreased (almost abolished) compared to WT mice (Table 1), further demonstrating the importance of Bcrp (abcg2) in sulfasalazine disposition. Additional studies are planned to better understand the contribution of intestinal secretion and hepatic biliary excretion of unchanged sulfasalazine. It is important to recognize that azoreduction of sulfasalazine by intestinal flora located in the distal intestine is the major contributor of metabolic clearance for this drug.[5,6] In the present study, sulfapyridine (one of the major metabolites of sulfasalazine) was much higher in the plasma of wild-type mice compared to Bcrp1 $(abcg2)^{-/-}$ KO after a single oral dose of sulfasalazine (20 mg/kg) (data not shown). This finding suggests that sulfasalazine transit time in the WT small intestine is more rapid due in part to Bcrp1 (abcg2) expression on the brush

(27) Elkind, N. B.; Szentpetery, Z.; Apati, A.; Ozvegy-Laczka, C.; Varady, G.; Ujhelly, O.; Szabo, K.; Homolya, L.; Varadi, A.; Buday, L.; Keri, G.; Nemet, K.; Sarkadi, B. Multidrug transporter ABCG2 prevents tumor cell death induced by the epidermal growth factor receptor inhibitor Iressa (ZD1839, Gefitinib). *Cancer Res.* **2005**, *65*, 1770−7.

border of intestinal enterocytes, thereby facilitating its conversion by bacterial flora in the distal intestine to sulfapyridine, which in turn is highly absorbed from the intestine into the blood. On the other hand, sulfapyridine plasma concentration was dramatically reduced in Bcrp1 (abcg2)$^{-/-}$ KO (data not shown). In support of this concept, C_{max} for sulfapyridine was 5-fold higher in WT compared to Bcrp (abcg2)$^{-/-}$ KO (data not shown). Interestingly, extrapolated C_0 of sulfasalazine in Bcrp1 (abcg2)$^{-/-}$ KO was 7-fold higher than that in the WT mice after a single intravenous dose (5 mg/kg) of the parent drug (Table 2). This difference in C_0 between the Bcrp1 (abcg2)$^{-/-}$ KO and the WT mice was not due to protein binding since protein binding of sulfasalazine was the same (98% data not shown) in both animals and suggested that the Bcrp1 (abcg2)$^{-/-}$ KO mice have a smaller volume of distribution of sulfasalazine than the WT mice. Our finding is in agreement with published data[14,19] in which nitrofurantoin and the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) C_0 concentrations in Bcrp1 (abcg2)$^{-/-}$ KO mice were higher than those in WT mice after single iv doses of these substrates. Moreover, the observed change (decrease) in V_d may help to explain why sulfasalazine half-life (pharmacokinetic-dependent variable on CL_s/V_d) is only moderately decreased in the abcg2 KO mice.

Surprisingly, our results suggest that P-gp does not play a significant role in the bioavailability of sulfasalazine (Figure 2 and Tables 1 and 2). Interestingly, estimated C_0 of sulfasalazine (single intravenous dose 5 mg/kg) was the same in mdr1a$^{-/-}$ and WT mice (Figure 2 and Table 2). Accordingly, P-gp does not appear to play a significant role in sulfasalazine disposition (Figure 2, Tables 1 and 2). Our data suggest that sulfasalzine may be an excellent marker probe substrate for intestinal BCRP (ABCG2) activity and that BCRP (ABCG2) may potentially influence the intestinal transit time of sulfasalazine. Therefore, we suggest the hypothesis that sulfasalazine may represent a dual probe for both BCRP (ABCG2) for intestinal drug absorption phenotype and metabolism of sulfapyridine to *N*-acetylsulfapyridine by *N*-acetyltransferase 2.[28,29] Moreover, these findings may also explain the very low absorption of sulfasalazine in the gastrointestinal tract observed clinically and may represent a mechanism to selectively deliver pharmaceuticals to the large intestine. However, it should be noted that the findings in this report relate to murine Bcrp (abcg2). It is unclear whether these observations may be translatable to human drug absorption; nevertheless, for other known BCRP substrates such as topotecan, there appears to be a pretty strong association between murine and human ABCG2 with respect to the absorption of compounds that display less than optimal physical chemical properties.[30,31]

In conclusion, we show that sulfasalazine is efficiently transported by murine bcrp (abcg2) in vivo. Given the high DNA sequence homology between human ABCG2 and murine abcg2 it is likely that human ABCG2 also plays a similar role in sulfasalazine absorption in humans.[30,31] Moreover, sulfasalazine has the potential to be used as a BCRP (ABCG2) specific in vivo probe and may aid in our understanding of the extent and relevance of BCRP (ABCG2) to the disposition of substrate drugs in humans and nonclinical species.

Acknowledgment. The authors would like to thank Dr. Richard Kim (Division of Clinical Pharmacology, Vanderbilt University School of Medicine) for his critical review and helpful suggestions for this manuscript. The authors wish to acknowledge the technical support of Betsy Galvan, Sheila Guppy, and Felicia Kpakima.

MP050113V

(28) Tanigawara, Y.; Kita, T.; Aoyama, N.; Gobara, M.; Komada, F.; Sakai, T.; Kasuga, M.; Hatanaka, H.; Sakaeda, T.; Okumura, K. N-Acetyltransferase 2 genotype-related sulfapyridine acetylation and its adverse events. *Biol. Pharm. Bull*. **2002**, *8*, 1058−62

(29) Yokogawa, K.; Nakaharu, T.; Ishizaki, J.; Ozaki, E.; Takeda, Y.; Mabuchi, H.; Matsushita, R.; Kimura, K.; Nakashima, E.; Ichimura, F.; Miyamoto, K. Kinetic phenotypic diagnosis of N-acetylation polymorphism in patients based on ratio of urinary metabolites of salicylazosulfapyridine. *Int. J. Pharm.* **2001**, *229*, 183−91.

(30) Jonker, J. W.; Buitelaar, M.; Wagenaar, E.; Van Der Valk, M. A.; Scheffer, G. L.; Scheper, R. J.; Plosch, T.; Kuipers, F.; Elferink, R. P.; Rosing, H.; Beijnen, J. H.; Schinkel, A. H. The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15649−54.

(31) Allen, J. D.; van Loevezijn, A.; Lakhai, J. M.; van der Valk, M.; van Tellingen, O.; Reid, G.; Schellens, J. H. M.; Koomen, G. J.; Schinkel, A. H. 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*. **2002**, *1*, 417−25.