Oral Sulfasalazine as a Clinical BCRP Probe Substrate: Pharmacokinetic Effects of Genetic Variation (C421A) and Pantoprazole Coadministration

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ABSTRACT: This study evaluated the utility of oral sulfasalazine as a probe substrate for Breast Cancer Resistance Protein (BCRP; ABCG2) activity by assessing the impact of genetic variation or coadministration of an inhibitor (pantoprazole) on plasma and urine pharmacokinetics of sulfasalazine and metabolites. Thirty-six healthy male subjects prescreened for *ABCG2* 421CC (reference activity), CA, and AA (lower activity) genotypes ($N = 12$ each) received a single 500 mg oral dose of enteric coated sulfasalazine alone, with 40 mg pantoprazole, or with 40 mg famotidine (gastrointestinal pH control) in a 3-period, single fixed sequence, crossover design. No significant difference in sulfasalazine or metabolite pharmacokinetics in 421AA or CA compared to 421CC subjects was found; however, high inter-subject variability was observed. Geometric mean (95% CI) sulfasalazine plasma $AUC_{(0-\infty)}$ values were 32.1 (13.2, 78.1), 16.8 (7.15, 39.6) and 62.7 (33.4, 118) μg h/mL, and C_{max} were 4.01 (1.62, 9.92), 1.70 (0.66, 4.40), and 6.86 (3.61, 13.0) μg/mL for CC, CA, and AA subjects, respectively. Pantoprazole and famotidine did not affect sulfasalazine pharmacokinetics in any genotypic cohort. These results suggest that the pharmacokinetics of oral, enteric-coated 500 mg sulfasalazine are not sufficiently sensitive to *ABCG2* genetic variation or inhibitors to be useful as a clinical probe substrate of BCRP activity. © 2009 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 99:1046–1062, 2010

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INTRODUCTION

Characterization of drug interaction potential is an important part of the risk-benefit assessment of new drugs in development. Perturbation of cytochrome P450 (CYP) and/or drug transporter activity is a central mechanism underlying many drug interactions that frequently requires *in vivo* clinical evaluation.[1,2] The validation of

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CYP-specific probe drugs providing quantitative assessments of enzyme activity *in vivo* has led regulators to accept the extrapolation of clinical probe drug interaction data of other drugs that are eliminated by the same CYP pathway. For example, the pharmacokinetic results of a drug interaction study with midazolam, a probe for CYP3A activity, allows categorization of the CYP3A inhibition by an investigational compound as a weak, moderate, or strong. This information, along with *in vitro* data, can provide the basis for a rational approach to prediction and assessment of drug interactions and reduce the requirement to conduct additional drug interaction studies with other known CYP3A substrates.[1]

The role of drug transporters in drug interactions is increasingly recognized.[2,3] However, the prediction of transporter-mediated interactions on a qualitative or quantitative basis remains a significant challenge, largely due to the lack of specific probe drugs. This is highlighted in the recent FDA Drug Interaction guidance that includes extensive information on P-glycoprotein (P-gp), the most widely studied transporter to date, but little information on other transporters.[1] Breast Cancer Resistance Protein (BCRP) is a member of the ATP Binding Cassette G (ABCG) family that has recently emerged as an important drug transporter for the disposition of a number of drugs, including tyrosine kinase inhibitors, topoisomerase 1 inhibitors and HMG-Co reductase inhibitors.[4,5] BCRP is constitutively expressed in healthy tissues including the intestine, liver, blood–brain barrier, breast, and placenta, as well as in tumor tissue, where it is one mechanism contributing to multidrug resistance.[4,5] BCRP in gastrointestinal enterocytes can serve as a barrier to drug absorption by pumping substrates back into the intestinal lumen, while the protein expressed at the hepatocyte canalicular membrane contributes to drug and metabolite elimination by pumping substrates out of the hepatocyte and into the bile. The combined result of intestinal and liver BCRP activity is lower systemic exposures of substrate drugs.[6] Several nonsynonymous single nucleotide polymorphisms (SNPs) in the human *ABCG2* gene have been associated with reduced BCRP transport activity, including C421A, C376T, G34A, T1291C, and T623C.[5] C421A, which results in a substitution of lysine for glutamine (Q141K) in the BCRP protein has been associated with increased drug exposure *in vivo* in man. The plasma exposures of oral topotecan and rosuvastatin were shown to be 1.4- and 1.8-fold higher, respectively, in patients heterozygous for the 421A variant than in subjects homozygous for wild-type 421C.[7,8] Based on these observations, genetic variation in *ABCG2* may contribute to the inter-subject variability in the pharmacokinetics of drugs that are BCRP substrates.

Characterizing the potential influence of *ABCG2* genetics or BCRP efflux activity on drug disposition is currently challenging due to the lack of a safe, well-characterized clinical probe substrate.[9] An ideal probe drug would be specific for BCRP and have easily measurable pharmacokinetic parameters that are sensitive to changes in BCRP function resulting from genetic variation or coadministration with a BCRP inhibitor. In addition, the ideal probe should undergo minimal metabolism, have a wide safety margin, and be commercially available in countries where clinical trials are conducted. Sulfasalazine, a marketed drug for the treatment of rheumatoid arthritis and ulcerative colitis, has the potential to be a human BCRP probe substrate based on published *in vitro* and *in vivo* animal studies.[10–15] Sulfasalazine has low permeability *in vitro* due to efflux transporters[10] and displays marked drug resistance in cells over expressing BCRP.[11,12] The primary mechanism underlying the low sulfasalazine absorption observed *in vivo* has been attributed to BCRP, as the plasma $AUC_{(0-\infty)}$ of the drug in Bcrp1 knockout mice was 111-fold higher than in Bcrp1 wild-type mice after oral administration and 13-fold higher after intravenous administration.[13] Sulfasalazine appears to be a specific probe for BCRP as it is not a substrate for P-gp *in vitro*[14,15] or in P-gp-knockout mice.[13] Sulfasalazine is not metabolized by CYP enzymes, but it is metabolized by reductase enzymes of colonic bacteria to 5-aminosalicylic acid (ASA) and sulfapyridine (SP), which in turn are absorbed into the systemic circulation[16] (see Study Design Considerations Section for more details of sulfasalazine metabolism).

The objective of this clinical study was to evaluate the pharmacokinetics of orally dosed sulfasalazine as a potential probe substrate of BCRP-mediated transport in healthy subjects *in vivo*. In addition, the effects of a known BCRP inhibitor on sulfasalazine pharmacokinetics were evaluated in the healthy subjects

by coadministration of oral pantoprazole (PPZ). Considering the effect of pantoprazole on gastric pH, each subject was also coadministered famotidine (FAM) and sulfasalazine as a gastrointestinal pH control.

MATERIALS AND METHODS

Study Design Considerations

Study Population and Sulfasalazine Dose Selection

The decision to conduct this study in an East Asian population (ethnic Chinese) was based on the higher frequency of the *ABCG2* C421A polymorphism in Chinese,[4] which would allow recruitment of adequate numbers of subjects of all three genotypes. The study was restricted to healthy men because sex differences in BCRP activity and/or expression have been reported.[17] A 500 mg dose of sulfasalazine was selected to minimize gastrointestinal adverse events (e.g., vomiting), to provide an acceptable safety margin should a substantial increase in systemic exposure occur when coadministered with the BCRP inhibitor, and to avoid reported nonlinear (less than dose proportional) pharmacokinetics at high doses.[18]

Selection of BCRP Inhibitors

Pantoprazole and famotidine are marketed drugs for the treatment of ulcers and gastroesophageal reflux disease (GERD). The use of pantoprazole as an inhibitor of BCRP-mediated drug transport is supported by studies demonstrating that pantoprazole inhibits the transport of a number of BCRP substrates *in vitro*,[19] that intravenous pantoprazole decreases the clearance of the BCRP substrate methotrexate by 1.7-fold in Bcrp1 wild-type, but not knock-out mice,[20] and that pantoprazole significantly improves brain penetration of imatinib (a BCRP substrate) in Bcrp1 wild-type mice.[21] One possible confounding issue with the use of 40 mg pantoprazole as a clinical BCRP inhibitor is that the drug's main pharmacological action is to inhibit gastric hydrogen/potassium adenosine triphosphatase, resulting in increased gastric pH.[22] Sulfasalazine is reported to have low aqueous solubility, particularly in acidic conditions, but little information is available on how increased gastric pH may affect sulfasalazine disintegration, dissolution and absorption. In addition, a recent *in vitro* study indicates that an increase in pH reduces the functionality of BCRP.[23] Given these observations, a third treatment arm was added to evaluate coadministration of sulfasalazine with 40 mg famotidine, a histamine H_2 receptor antagonist that also increases gastric and upper duodenal pH, and serves as a control for gastrointestinal pH. *In vitro* studies conducted in our laboratory using [^{3}H]-cimetidine as a probe BCRP substrate and MDCKII-BCRP cell monolayers[24] revealed that famotidine was a much less potent inhibitor of BCRP (IC_{50} value of $62 \pm 8.3\,\mu M$; mean $\pm$ SD) compared to pantoprazole (IC_{50} of $4.9 \pm 0.42\,\mu M$).

Sulfasalazine Metabolism

In healthy subjects, approximately 10–20% of the sulfasalazine dose is absorbed from the upper small intestine. When unabsorbed sulfasalazine reaches the colon, it is cleaved by bacterial enzymes from multiple bacterial species to two metabolites: sulfapyridine and 5-aminosalicylic acid (mesalamine).[25] The extent of metabolism depends on the activity of the intestinal flora and intestinal transit time.[16] In fact, the appearance of the sulfapyridine metabolite in the systemic circulation has been used as a measure of small intestine transit time.[26] Sulfapyridine is nearly completely absorbed and then excreted in the urine as acetyl- or glucuronide-conjugate metabolites. In contrast, 5-aminosalicylic acid is poorly absorbed (10–30%), with the majority excreted unchanged in feces and the rest excreted in the urine as an acetylated metabolite. Elimination of the sulfasalazine metabolites is strongly influenced by *N*-acetyltransferase activity.[16] Therefore, to better understand the importance of *ABCG2* SNPs on the absorption of sulfasalazine, the plasma and urinary concentrations of sulfapyridine, *N*-acetylsulfapyridine, and 5-aminosalicylic acid along with the subject's NAT2 genotype were determined.

Clinical Study

Subjects

Thirty-six healthy male subjects (age 21–45 years) on no other medication were recruited for this study. Ethnicity was defined through self-declaration by each subject and was consistent over three generations. All subjects underwent an initial screening assessment following collection of written informed consent and provided a blood sample

for genotyping. A second screening assessment within 28 days of the first dose was conducted and included a medical history, physical examination, blood pressure and ECG measurements as well as clinical laboratory tests. Exclusion criteria included a positive urine drug test, past or current history of excessive alcohol or illicit drug use, recent participation in another research trial (i.e., within 30 days prior to the first dose of sulfasalazine), use of any prescription or nonprescription drugs, vitamins, herbal and dietary supplements or grapefruit-containing products within 7 days or 5 half-lives prior to the first dose sulfasalazine or during the clinical phase of the study, regular use of tobacco- or nicotine-containing products within 3 months of screening visit, and any preexisting conditions that would interfere with normal gastrointestinal anatomy or motility, hepatic and/or renal function.

Study Design

This open-label, 3-period, single fixed sequence, crossover study was approved by the ethics review board at National University of Singapore. Subjects were genotyped for *ABCG2* C421A polymorphism prior to entry into the study and assigned to one of three cohorts: 421CC, 421CA, or 421AA ($n = 12$ per group). Each subject received three treatments with a 1 week between-treatment washout period as follows: Treatment A—Sulfasalazine 500 mg (Salazopyrin-EN®, Pfizer, Singapore, Singapore); Treatment B—Sulfasalazine 500 mg + Pantoprazole 40 mg (Controloc®, Altana Pharma, Singapore, Singapore); Treatment C—Sulfasalazine 500 mg + Famotidine 40 mg (Pepcidine®, Merck Sharp & Dohme, Singapore, Singapore). Subjects were admitted to the unit the evening prior to each day of dosing and stayed until after the last pharmacokinetic sample was obtained. Study drug(s) were administered with 240 mL of water following a 10-h overnight fast. Subjects remained fasted until 4 h after dosing when a meal was provided. The composition of the lunch meal was identical on each of the three study days. Subjects not completing all planned study related activities were not replaced; however, pharmacokinetic results from subjects who did not complete all phases of the study were included in the final analysis.

Blood samples (2 mL) for the determination of sulfasalazine, sulfapyridine and 5-aminosalicylic acid plasma concentrations were collected in EDTA-containing tubes prior to each dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 10, 12, 16, 24, 30 h after each dose. A predose urine sample was collected prior to dosing and subjects were asked to empty their bladders before dosing. Thereafter, all urine passed over the next 30 h was collected into separate preweighed containers over the following collection intervals: 0–12, 12–24, 24–30 h. All plasma and urine samples were stored frozen at −70°C until assayed.

Genotyping of ABCG2 and NAT2 Polymorphisms

A whole blood sample (5 mL) for genotyping was obtained from each subject. The genomic DNA extracted from peripheral leukocytes by standard methods was used as a template in amplification of the fragments encompassing the following SNPs of *ABCG2* gene: G34A, C376T, C421A, T623C, and T1291C residing in exons 2, 4, 5, 6, and 11, respectively, as described previously.[27] Genotyping for the *N*-acetyltransferase 2 (*NAT2*) gene was completed to determine the relationship of *NAT2* genotype to the metabolism of sulfapyridine. Two primer pairs (NAT2aF: 5′-catggagttgggcttagag-3′, NAT2aR: 5′-ctttggcaggagatgagaa-3′; NAT2bF: 5′-gctgggtctggaagctcctc-3′, NAT2bR: 5′-ttgggtgatacatacacaaggg-3′) were designed using Lasergene DNASTAR software (DNASTAR, Inc., Madison, WI) to generate overlapping fragments that encompass the polymorphism sites for defining the various alleles. The amplifications for both fragments were performed in a total volume of 50 μL containing 1X Master Mix (Promega, Madison, WI), 0.2 mM of each primer (Sigma-Proligo, St. Louis, MO) and 100 ng of DNA. Following an initial predenaturation step at 95°C for 5 min, the reactions were cycled 35 times through denaturation at 95°C for 1 min, annealing at 54°C for 1 min and extension at 72°C for 1 min. The reactions were terminated by an additional extension step at 72°C for 10 min. The sequencing was carried out as described previously.[27] Classification of NAT2 activity based on genotype was defined as follows: Slow acetylators—*6B/*6B, *6A/*6A, *6A/*7B, *7B/*7B, *5C/*7B, *5C/*6A; Intermediate acetylators—*4/*6A, *4/*7B, *4/*5D, *4/*6B; *4/*5C; Fast acetylators—*4/*4, *4/*12A, *13/*13.[28,29]

Bioanalytical Methods

The concentration of sulfasalazine, sulfapyridine, 5-aminosalicylic acid, and pantoprazole in plasma samples and standards was determined

simultaneously by high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) using [$^{13}C_4$]-sulfasalazine (GlaxoSmithKline, Inc. Stevenage, United Kingdom), [$^{2}H_4$]-sulfapyridine (GlaxoSmithKline, Inc.), [$^{13}C_4$]-5-aminosalicylic acid, and deuterated pantoprazole (Toronto Research Chemicals, Inc., North York, Canada) as the internal standards. Sulfasalazine, sulfapyridine, and 5-aminosalicylic acid were obtained from Sigma-Aldrich (St. Louis, MO) and pantoprazole was obtained from Toronto Research Chemicals, Inc. Using a 50 μL sample, plasma proteins were precipitated by the addition of 150 μL of acetonitrile containing internal standard; the concentration of the internal standard was 250 ng/mL for [$^{13}C_4$]-sulfasalazine, [$^{2}H_4$]-sulfapyridine and deuterated pantoprazole, and 500 ng/mL for [$^{13}C_4$]-5-aminosalicylic acid. Following mixing by vortexing for 2 min precipitant was removed by centrifugation (5 min at 3000*g*). The resulting supernatants (100 μL) were diluted with 300 μL of HPLC water. HPLC was performed on a Shimadzu LC-10A HPLC system (Shimadzu Corporation, Kyoto, Japan). Chromatography was performed on a Varian Polaris C18-A 3 μm, 2.1 mm × 50 mm column (Varian, Inc., Palo Alto, CA) at a flow rate of 0.4 mL/min. The mobile phase consisted of 2 solvents: (A) HPLC water with 0.1% heptafluorobutyric acid and (B) acetonitrile with 0.1% heptafluorobutyric acid using a linear gradient over 2 min starting with a mobile phase of 95%A:5%B and ended with a mobile phase of 10%A:90%B. Samples (4 μL injection volume) were analyzed by positive ion turbo ionspray LC/MS/MS with a PE/Sciex API 4000 (Applied Biosystems/MDS Sciex, Concord, Canada). The calibration range for sulfasalazine, sulfapyridine, 5-aminosalicylic acid, and pantoprazole was 20–10,000 ng/mL, with the lower limit of quantification being 20 ng/mL for each analyte. The precursor and product ion (*m*/*z*) pairs were: sulfasalazine 399/223, sulfapyridine 250/156, 5-aminosalicylic acid 154/80, and pantoprazole 384/128. Raw data were analyzed with PE/Sciex software Analyst 1.4.1. SMS 2000 (version 1.6, GlaxoSmithKline, Inc.) was used to calculate peak area ratios and to construct the calibration lines from which concentrations of unknowns were interpolated. Performance of the method, as assessed by sulfasalazine, sulfapyridine, 5-aminosalicylic acid, and pantoprazole concentrations in quality controls samples (60, 800, 8000 ng/mL), showed that the average within-run precision (coefficient of variation, CV%) was less than or equal to 10.0%. The between-run precision CV% was less than or equal to 4.7%. The accuracy for the lowest calibration standard for each analyte was: 99.7% for sulfasalazine, 100.0% for sulfapyridine, 100.2% for 5-aminiosalicylic acid, and 100.4% for pantoprazole.

The urine concentrations of sulfasalazine, sulfapyridine, and acetylsulfapyridine were determined by HPLC with ultraviolet detection (HPLC/UV), with a lower limit of quantitation of 0.1 μg/mL. Urine concentrations of 5-aminosalicylic acid were expected to be low and therefore not determined. For sulfasalazine, a 100 μL urine aliquot was combined with 10 μL of the internal standard furazolidone (100 μg/mL; Sigma-Aldrich Co, Singapore, Singapore) and vortexed. Next 200 μL of pH 1.8 buffer was added to adjust urine pH to around 2, followed by 1.7 mL of *tert*-butyl methyl ether extraction solvent. Samples were vortexed for 5 min and then centrifuged at 10,000 rpm for 5 min. The supernatant was evaporated under nitrogen and then reconstituted with 100 μL of mobile phase, vortexed, and centrifuged. A 25 μL sample was injected into the HPLC system which consisted of an Agilent 1050 series pump and online degasser and an 1100 series auto-sampler and Variable Wavelength Detector (Agilent Technologies, Inc., Santa Clara CA). Separation was achieved with a Gemini C18, 5 μm, 110 Å, (4.6 mm × 150 mm) column (Phenomenex, Torrance, CA) connected to an analytical guard cartridge at an oven temperature of 40°C. The mobile phase consisted of 81.5% 20 mM disodium phosphate with 0.2% triethylamine (pH adjusted to 10 with phosphoric acid) and 18.5% acetonitrile delivered at a flow rate of 1 mL/min. Sulfasalazine was detected at a wavelength of 360 nm. Calibration standards over the range of 0.1–50 μg/mL and QC samples (0.25, 2.5, 25 μg/mL) were assayed along with each set of samples. The inter-day and intra-day precision CV% ranged from 3.4% to 4.6% and 2.4% to 2.8%, respectively, and precision for the lowest calibration standard was 8.6%. Accuracy for the method was 100% to 107% (inter-day variability), 94% to 101% (intra-day variability), and 99.3% for the lowest calibration standard.

For sulfapyridine and *N*-acetylsulfapyridine, a 50 μL urine aliquot was combined with 10 μL of the internal standard sulfadiazine (500 μg/mL) and vortexed. Next 150 μL of pH 7.4 buffer was added to adjust urine pH to 7.2–7.3 to ensure consistent extraction of the basic metabolites, followed by 1.6 mL of *tert*-butyl methyl ether

extraction solvent. Samples were vortexed for 5 min and then centrifuged at 10,000 rpm for 5 min. The supernatant was transferred to another tube and evaporated. The dried supernatant was reconstituted with 50 μL of mobile phase, vortexed, centrifuged and then 20 μL was injected into the Agilent HPLC system 1050 with an 1100 series Auto-sampler and Variable Wavelength Detector (Agilent Technologies, Inc.). The metabolites were eluted from a Hypersil BDS C18, 5 μm, 4.6 mm × 200 mm column (Thermo Hypersil-Keystone, Astmoor, UK) and an analytical BDS C18, 5 μm guard column (maintained at room temperature) by a mobile phase consisting of 84% 5 mM disodium phosphate with 0.067% tetra-*n*-butylammonium chloride (pH adjusted to 7.4 with phosphoric acid) and 16% methanol at a flow rate of 1 mL/min. The UV detection wavelength was 310 nm. Calibration standards of sulfapyridine and *N*-acetylsulfapyridine (both obtained from Sigma, Singapore, Singapore) over the range of 0.1 to 25 μg/mL and QC samples (0.25, 2.5, 25 μg/mL) were assayed along with each set of samples. For sulfapyridine, the inter-day and intra-day precision CV% ranged from 2.8% to 10.2% and 1.9% to 2.7%, respectively, and precision for the lowest calibration standard was 9.1%. Accuracy for the method was 88.7–99% (inter-day variability), 88.9–104% (intra-day variability) and 97.8% for the lowest calibration standard. For *N*-acetylsulfapyridine, the CV% ranged from 4.3% to 6.6% (inter-day) and 2.5% to 4.8% (intra-day) and precision for the lowest calibration standard was 7.3%. Accuracy was 96.3–104% (inter-day) and 93.3–108% (intra-day) and 105% for the lowest calibration standard.

Pharmacokinetic Evaluation

The plasma pharmacokinetic parameters of sulfasalazine, sulfapyridine, and 5-aminosalicylic acid were estimated by noncompartmental methods with WinNonlin Version 4.1 (Pharsight, Mountain View, CA) using actual pharmacokinetic sampling time. The maximum observed drug concentration (C_{max}) and the first time of its occurrence (T_{max}) were taken directly from the concentration-time profile. The area under the concentration-time profile from zero time to infinity ($AUC_{(0-\infty)}$) was calculated for sulfasalazine and sulfapyridine using the linear up/logarithmic down trapezoidal method ($AUC_{(0-last)}$) and extrapolation to infinite time by the addition of C_{last}/λ_z, where λ_z is the apparent terminal phase elimination rate constant estimated by linear regression of the logarithmically transformed concentration data. A minimum of three terminal phase concentration values were used to estimate λ_z. Ratios of metabolite-to-parent ($AUC_{(0-t\ or\ \infty)}$) values were calculated based on molar concentrations using the molecular weights (MW) of sulfapyridine (249.3), acetylsulfapyridine (291.3), and sulfasalazine (398.4).

The total urinary recovery (Ae_∞) of sulfasalazine, sulfapyridine or *N*-acetylsulfapyridine was calculated as the sum of the amount of the respective compound excreted in each urine collection interval. The percentage of the dose that was excreted as unchanged sulfasalazine in urine (fe) was calculated as $fe = Ae_\infty/Dose \times 100$ and the apparent renal clearance (CL_{renal}) of sulfasalazine was calculated as $CL_{renal} = Ae_\infty/AUC_{(0-\infty)}$. The percentage of the dose that was excreted as sulfapyridine or *N*-acetylsulfapyridine, in urine (fe) was calculated as $fe = Ae_\infty/Dose \times MW_{sulfasalazine}/MW_{metabolite} \times 100$, where MW is the molecular weight of sulfasalazine or metabolite as given above.

Statistical Analyses

Statistical analyses were performed with SAS (version 8.2, Cary, NC). The geometric mean and 95% confidence intervals were calculated by genotypic cohort and treatment for each pharmacokinetic parameter, except T_{max} for which median and range were calculated. Two-way analysis of variance (ANOVA) was used to compare log-transformed pharmacokinetic parameters between the subjects among genotypic cohorts (CC, CA, AA) and within the subjects among treatments (SFZ Alone, SFZ + PPZ, SFZ + FAM) and calculate geometric least squares (GLS) mean cohort ratios and treatment ratios and associated 90% confidence intervals.

RESULTS

Subject Demography and Pharmacogenetics

Subject demographic characteristics are presented by *ABCG2* 421 genotypic cohort in Table 1. All subjects were ethnic Chinese and similar age and body weight ranges were noted among cohorts. Retrospective genotyping for other *ABCG2* and *NAT2* alleles was also

Table 1. Summary of Demographic and Genotype Data by *ABCG2* 421 Cohort

	ABCG2 421 Genotype		
	CC	CA	AA
Number of subjects			
SFZ Alone	12	12	12
+Pantoprazole	10	12	12
+Famotidine	9	12	12
Body weight (kg)[a]	72.8 ± 7.3 (58.9–86.7)	68.9 ± 8.0 (57.5–81.9)	70.3 ± 6.6 (56.5–79.3)
Age (year)[a]	29.5 ± 7.2 (23–43)	36.3 ± 8.5 (24–45)	30.2 ± 6.6 (21–42)
ABCG2 34 genotype (*N*)			
GG	6/5/4[b]	4	12
GA	4/3/3[b]	7	0
AA	2/2/2[b]	1	0
NAT2 activity[c] (*N*)			
Fast	1/1/0[b]	3	0
Intermediate	5/4/4[b]	7	8
Slow	6/5/5[b]	2	4

[a]Weight and age are presented as mean ± SD (range) for $N = 12$.
[b]Period 1/Period 2/Period 3.
[c]NAT2 activity defined as follows: Slow—*6B/*6B, *6A/*6A, *6A/*7B, *7B/*7B, *5C/*7B, *5C/*6A; Intermediate—*4/*6A, *4/*7B, *4/*5D, *4/*6B; *4/*5C; Fast—*4/*4, *4/*12A, *13/*13.

completed. The distribution of G34A genotypes and NAT2 activity are presented in Table 1. The majority of the subjects were 34GG ($N = 22$; 61%), with few being 34AA ($N = 3$; 8%); no subjects were both 421AA and 34AA or 34GA. Other variant *ABCG2* alleles were not observed and all subjects were *ABCG2* 376CC, 623TT, and 1291TT. Subjects were also classified as slow, intermediate, or fast acetylators based on *NAT2* genotyping (Tab. 1). As expected for this East Asian population, most subjects were intermediate ($N = 20$; 56%) or slow ($N = 12$; 33%) acetylators.

Sulfasalazine Pharmacokinetics

For Treatment A (sulfasalazine alone), all 36 subjects completed dosing and had evaluable pharmacokinetic parameters. Overall, 33 subjects completed all three treatment periods. Three subjects withdrew from the study for personal reasons: two subjects (genotype CC) completed only Treatment A and one subject (genotype CC) completed Treatments A and B. Data from all subjects was included in the pharmacokinetic analysis. The single dose of sulfasalazine was well-tolerated when given alone and with pantoprazole or famotidine. No serious adverse events were reported.

Mean plasma sulfasalazine concentration-time profiles following a single 500 mg oral dose from an enteric coated tablet given during Treatment A (sulfasalazine alone) are shown in Figure 1 and the pharmacokinetic parameters are summarized in Table 2. The absorption of sulfasalazine was delayed with a lag time of 2.5 h and T_{max} of 5–6 h. High inter-subject variability in sulfasalazine plasma and urinary pharmacokinetic parameters was observed both across and within genotypic cohorts (Tab. 2, Fig. 1), with $AUC_{(0-\infty)}$ ranging 81-, 66-, 21-fold for the CC, CA, and AA cohorts. No significant differences in sulfasalazine $AUC_{(0-\infty)}$, C_{max}, T_{max}, $T_{1/2}$, or % dose excreted unchanged in the urine were observed between the subjects among *ABCG2* 421 genotypic cohorts. Of note, two subjects in the 421CA cohort had extremely low concentrations of sulfasalazine ($AUC_{(0-\infty)}$ 1.4 and 1.8 μg h/mL compared to the median value of 16.8 μg h/mL). Exclusion of these two subjects in a subset analysis (data not shown) did not influence the statistical significance of the results, further confirming the lack of a significant gene dose effect for the CC and AA genotypes.

Fourteen subjects were either heterozygous carriers (seven in the 421CA cohort; four in the 421CC cohort) or homozygous carriers (one in the 421CA cohort; two in the 421CC cohort) of the G34A SNP (Tab. 1). A summary of the sulfasalazine pharmacokinetic parameters by G34A polymorphism status indicated that

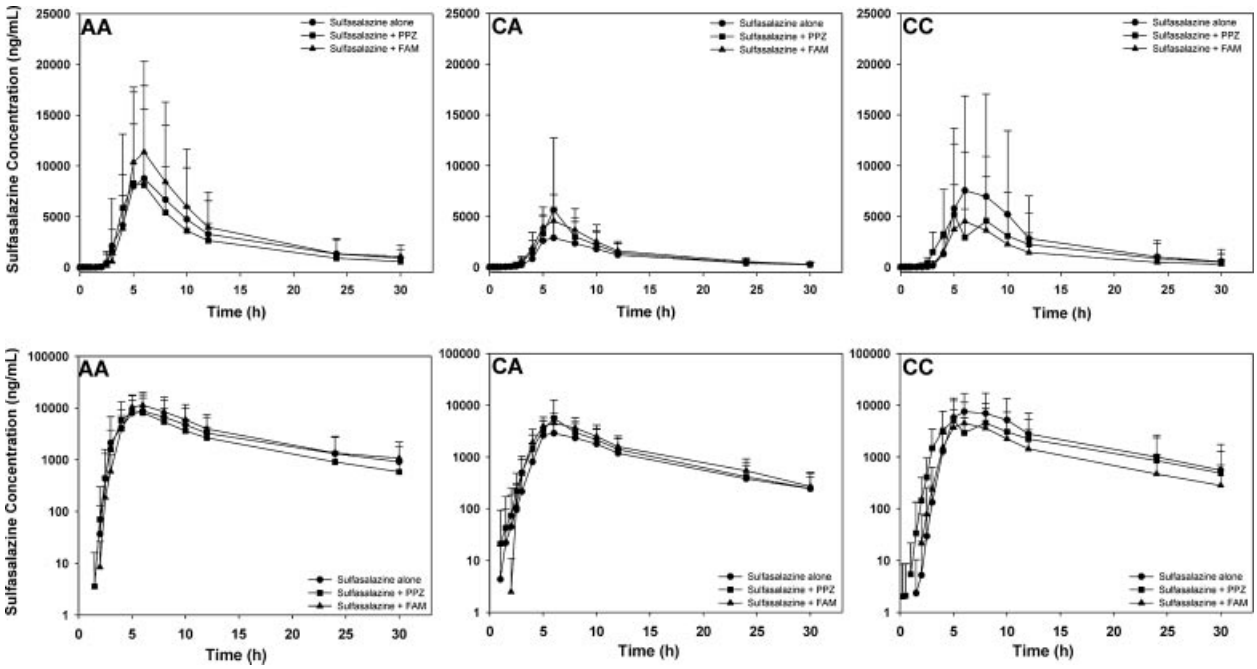

Figure 1. Mean (±SD) sulfasalazine plasma concentration-time profiles in *ABCG2* 421CC, CA, and AA genotype cohorts following oral administration of sulfasalazine alone or coadministered with pantoprazole (PPZ) or famotidine (FAM). Linear and log scales shown.

sulfasalazine $AUC_{(0-\infty)}$ was lower in 34AA subjects (geometric mean 8.85 μg h/mL; $n = 3$) than in 34GA (37.4 μg h/mL; $n = 11$) and 34GG subjects (36.0 μg h/mL, $n = 22$). The geometric mean C_{max} values were 1.09, 4.52, and 3.79 μg/mL for *ABCG2* 34GG, GA, and AA genotypes, respectively. This notably lower $AUC_{(0-\infty)}$ is unexpected as the G34A SNP has not been associated with increased BCRP function; generally this SNP has been associated with no change or a decrease in BCRP activity.[4] No consistent trends in sulfasalazine $AUC_{(0-\infty)}$ across G34A/C421A genotype groups were observed.

Metabolite Pharmacokinetics

Mean plasma sulfapyridine concentration-time profiles during Treatment A (sulfasalazine alone) are shown in Figure 2 and the pharmacokinetic parameters are summarized in Table 3. Sulfapyridine had a lag time of approximately 4 h and T_{max} of 10–16 h, which were both longer than the respective sulfasalazine parameters. The half-life and $AUC_{(0-\infty)}$ could not be accurately estimated for several subjects; therefore, $AUC_{(0-t)}$ is also presented for sulfapyridine. As with sulfasalazine, high inter-subject variability was noted in the plasma pharmacokinetic parameters and no significant differences were noted in the sulfapyridine $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, C_{max}, T_{max}, $T_{1/2}$, or % dose excreted unchanged in the urine among the *ABCG2* 421 genotypic cohorts (Tab. 3). Molar ratios of the metabolite $AUC_{(0-t)}$-to-parent $AUC_{(0-\infty)}$ were calculated to adjust for the highly variable sulfasalazine exposures. Subjects in the 421AA cohort tended to have lower sulfapyridine exposures relative to sulfasalazine (AUC_{SP}/AUC_{SFZ} ratio = 0.548), consistent with lower BCRP activity, whereas subjects in the 421CC cohort tended to have higher sulfapyridine exposures relative to sulfasalazine (AUC_{SP}/AUC_{SFZ} ratio = 3.55). However, inter-subject variability in the AUC ratios was high and no significant differences in these ratios were noted among genotypic cohorts. Across all study cohorts, no consistent, significant relationship was observed between sulfapyridine pharmacokinetic parameters and *NAT2* genotype (data not shown).

Plasma concentrations of the 5-aminosalicylic acid metabolite were not detected in one-third of the subjects. In subjects where 5-aminosalicylic acid was detected, concentrations were low and only measurable at a few sample times which

Table 2. Sulfasalazine Plasma and Urine Pharmacokinetic Parameters in *ABCG2* 421CC, CA, and AA Genotype Cohorts and between Genotype Comparisons Following a Single Oral Dose of 500 mg Sulfasalazine to Healthy Male Subjects

	ABCG2 421 Genotype			Ratio of Geometric LS Means [90% CI]	
	CC ($N = 12$)	CA ($N = 12$)	AA ($N = 12$)	CA vs. CC	AA vs. CC
$AUC_{(0-\infty)}$ (μg h/mL)	32.1 (13.2, 78.1) [247]	16.8 (7.15, 39.6) [227]	62.7 (33.4, 118) [129]	0.52 [0.22, 1.26]	1.95 [0.81, 4.71]
C_{max} (μg/mL)	4.01 (1.62, 9.92) [258]	1.70 (0.66, 4.40) [288]	6.86 (3.61, 13.0) [133]	0.43 [0.17, 1.06]	1.71 [0.69, 4.27]
T_{max} (h)	6.00 (4.03–10.0)	6.00 (4.00–12.0)	5.00 (4.00–8.00)	0.99 [0.81, 1.21]	0.82 [0.67, 1.00]
$T_{1/2}$ (h)	6.80 (6.04, 7.66) [19]	7.62 (6.83, 8.51) [17]	8.76 (7.85, 9.79) [18]	1.12 [0.99, 1.27]	1.29 [1.14, 1.46]
T_{lag} (h)	2.50 (1.00–4.00)	2.75 (0.75–8.00)	2.25 (1.50–4.00)	—	—
% Dose in urine	2.60 (1.11, 6.11) [226]	1.17 (0.537, 2.55) [187]	5.68 (3.28, 9.85) [106]	0.45 [0.19, 1.05]	2.18 [0.94, 5.08]

N, sample size in each genotype cohort.
Pharmacokinetic parameters shown as geometric mean (95% CI) and [CVb%] except T_{max} and T_{lag} which are presented as median (range).

precluded estimation of all pharmacokinetic parameters except T_{lag}, T_{max}, and C_{max}. These parameters are summarized in Supporting Information Table S1. No trend in relation to C421A genotype was observed and given the limited data, no statistical analyses were conducted.

Effect of Pantoprazole and Famotidine on Sulfasalazine and Sulfapyridine Pharmacokinetics

Figures 1 and 2 compare the concentration-time profiles of sulfasalazine and sulfapyridine, respectively, when sulfasalazine was administered alone or in combination with 40 mg pantoprazole or 40 mg famotidine. Figure 3 shows individual subject data, as well as box plots, for selected pharmacokinetic parameters. Statistical summaries of the pharmacokinetic parameters of sulfasalazine and sulfapyridine when coadministered with pantoprazole are given in Tables 4 and 5, respectively and when coadministered with famotidine are given in Supporting Information Tables S2 and S3, respectively. Although high inter-subject variability in pharmacokinetic parameters was observed, visual inspection of the plots and ANOVA did not reveal any consistent differences in sulfasalazine or sulfapyridine pharmacokinetic parameters when sulfasalazine was administered with pantoprazole or famotidine, compared to when it was administered alone.

DISCUSSION

Emerging data over the past decade have demonstrated the important role of transporters in drug disposition and as a potential source of drug interactions.[2,3] In contrast to CYP enzymes that have well-established probe drugs for assessment of drug interactions, few drugs have been evaluated as probes for drug transporters. Therefore, the objective of this clinical study was to evaluate the pharmacokinetics of orally dosed sulfasalazine as a potential probe substrate for BCRP. In addition, the effects of a BCRP inhibitor on sulfasalazine pharmacokinetics were evaluated by coadministration of oral pantoprazole.

Healthy male subjects with *ABCG2* 421CC, CA, and AA genotypes received a single 500 mg oral dose of enteric coated sulfasalazine in a 3-period, single fixed sequence, crossover design.

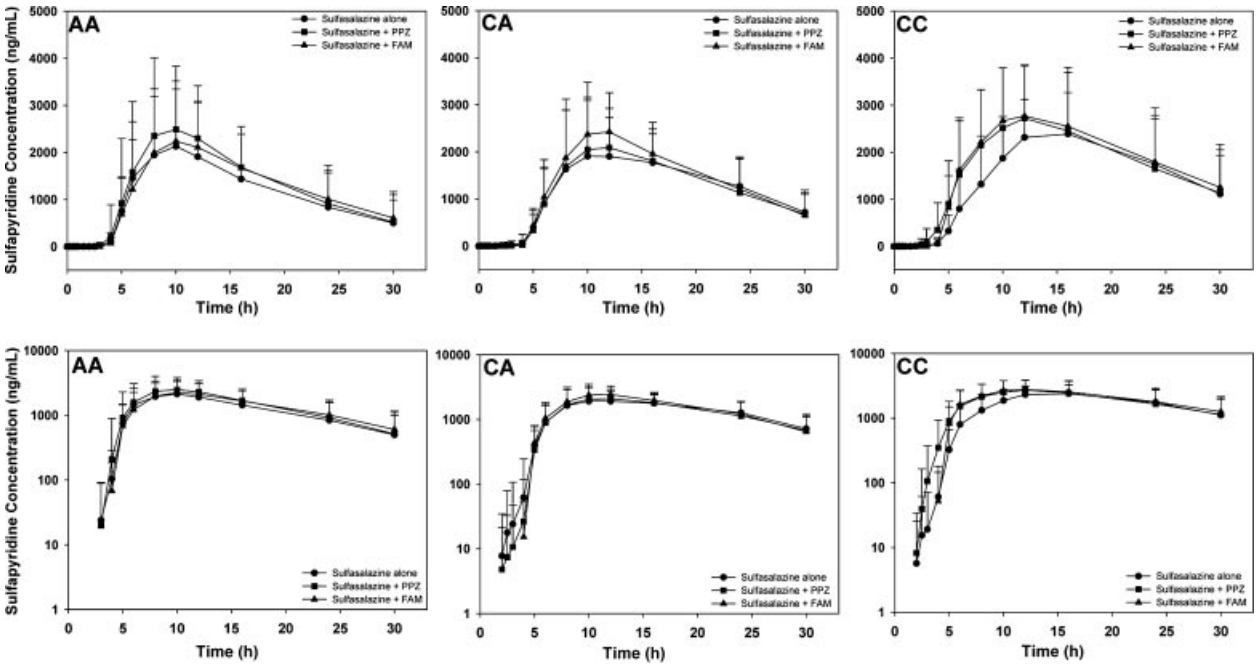

Figure 2. Mean (±SD) sulfapyridine plasma concentration-time profiles in *ABCG2* 421CC, CA, and AA genotype cohorts following oral administration of sulfasalazine alone or coadministered with 40 mg pantoprazole (PPZ) or 40 mg famotidine (FAM). Linear and log scales shown.

High inter-subject variability in sulfasalazine and metabolite pharmacokinetic parameters was observed in all genotypic cohorts and across all dosing periods. Although there was a trend for higher sulfasalazine exposure in the 421AA cohort across all three treatment periods (~2-fold higher geometric mean sulfasalazine $AUC_{(0-\infty)}$, C_{max}, and fe in 421AA compared to 421CC cohort), the confidence intervals were wide and overlapping and overall no significant differences in sulfasalazine or metabolite pharmacokinetic parameters among 421CC, CA, or AA genotypes were detected. In addition, no gene-dose effect was observed; the mean sulfasalazine exposure in the heterozygous 421CA cohort was lower (Treatment A), equal (Treatment B), or higher (Treatment C) than the 421CC cohort; however, the sulfasalazine exposure was lower in the 421CA cohort compared to the 421AA cohort across all three treatments. Sulfapyridine-to-sulfasalazine molar AUC ratios were calculated to account for inter-individual differences in sulfasalazine absorption. The sulfapyridine-to-sulfasalazine molar AUC ratios tended to be higher for the 421CC and CA cohorts (3.55 and 2.83, respectively for Treatment A) compared to the 421AA cohort (0.55 for Treatment A), but were not statistically significant. A similar trend was shown previously suggesting that greater sulfasalazine absorption yields less production of sulfapyridine by gut flora and a lower metabolite ratio in 421AA subjects compared to CC carriers.[30]

In the second part of this study, subjects received the same dose of sulfasalazine along with 40 mg pantoprazole (BCRP inhibitor) or with 40 mg famotidine (gastrointestinal pH control) to assess the effect of a BCRP inhibitor on the pharmacokinetics of sulfasalazine and its metabolites. Oral pantoprazole or famotidine did not affect sulfasalazine pharmacokinetics in subjects of any genotypic cohort, a surprising result given that pantoprazole intestinal concentrations were estimated to be 40 mM, (almost 10,000-fold higher than the *in vitro* IC value of 4.2 μM), and that plasma pantoprazole concentrations similar to those previously reported were achieved (data not shown).

Collectively, the results show that neither the *ABCG2* C421A polymorphism or coadministration with a BCRP inhibitor has a substantial effect on the pharmacokinetics in man of a sulfasalazine 500 mg dose administered as the enteric coated formulation. This lack of effect suggests that BCRP plays a minor role in the disposition of

Table 3. Sulfapyridine Plasma and Urine Pharmacokinetic Parameters in *ABCG2* 421 CC, CA, and AA Genotype Cohorts and between Genotype Comparisons Following a Single Oral Dose of 500 mg Sulfasalazine to Healthy Male Subjects

	ABCG2 421 Genotype			Ratio of Geometric LS Means [90% CI]	
	CC ($N = 12$)	CA ($N = 12$)	AA ($N = 12$)	CA vs. CC	AA vs. CC
$AUC_{(0-t)}$ (μg h/mL)	40.1 (29.3, 54.8) [53]	34.2 (27.9, 41.8) [33]	25.2 (14.7, 43.2) [102]	0.85 [0.55, 1.31]	0.63 [0.41, 0.97]
$AUC_{(0-\infty)}$ (μg h/mL)	52.2 (34.1, 80.0) [71] ($n = 11$)	45.3 (33.1, 62.0) [46] ($n = 10$)	29.6 (16.5, 53.2) [115]	0.75 [0.43, 1.29]	0.52 [0.30, 0.89]
C_{max} (μg/mL)	2.47 (1.96, 3.11) [38]	2.32 (1.85, 2.92) [37]	1.86 (1.12, 3.10) [94]	0.94 [0.64, 1.39]	0.76 [0.51, 1.11]
T_{max} (h)	16.0 (8.00–24.0)	12.0 (8.00–24.0)	10.0 (6.00–16.0)	0.90 [0.71, 1.14]	0.67 [0.53, 0.86]
$T_{1/2}$ (h)	10.6 (7.94, 14.2) [45] ($n = 11$)	9.08 (6.85, 12.0) [41] ($n = 10$)	7.80 (6.25, 9.73) [36]	0.92 [0.68, 1.23]	0.80 [0.60, 1.08]
T_{lag} (h)	4.00 (1.50–6.00)	4.00 (1.50–8.00)	4.00 (2.50–5.00)	—	—
% Dose in urine	4.64 (3.49, 6.15) [47]	3.95 (2.78, 5.62) [60]	3.33 (1.83, 6.05) [120]	0.85 [0.54, 1.34]	0.72 [0.46, 1.13]
$AUC_{(0-t)SP}/AUC_{(0-\infty)SFZ}$	3.55 (0.08–13.5)	2.83 (0.75–27.7)	0.548 (0.12–2.67)	1.63 [0.55, 4.82]	0.32 [0.11, 0.95]

N, sample size in each genotype cohort.
Pharmacokinetic parameters shown as geometric mean (95% CI) and [CVb%], except T_{max}, T_{lag} and AUC ratio which are presented as median (range).

sulfasalazine or that other transporters are able to compensate for loss of BCRP activity due to genetic deficiency or chemical inhibition. In addition, this study indicates that genetic variability in BCRP activity is not the major source of variability in sulfasalazine plasma and urine pharmacokinetics. Of note, the effect of genetic variation in *ABCG2* 421 on the pharmacokinetics of other BCRP substrates has been modest. For example, intravenous diflomotecan had threefold higher AUC in 421CA ($n = 1$) versus CC carriers,[31] topotecan had a 1.3-fold higher AUC in CA ($n = 2$) versus CC,[7] and rosuvastatin had a 1.8-fold higher AUC in a combined cohort of 421CA/AA versus CC.[8] No effect of the 421A genotype was observed for oral nitrofurantoin, lamivudine, pravastatin, pitavastatin, and intravenous irinotecan.[27,32–35]

During the preparation of this manuscript, two clinical studies evaluating sulfasalazine as a BCRP probe substrate and the influence of the C421A polymorphism were published.[30,36] In one study, Urquhart et al.[36] characterized sulfasalazine pharmacokinetics and the *in vivo* expression of BCRP along the human gastrointestinal tract following a 1000 mg sulfasalazine oral suspension dose to healthy male and female subjects. BCRP protein and mRNA expression had low variability across the subjects (1.8- and 2.7-fold, respectively), displayed no significant difference in duodenal expression in subjects with C421A or G34A polymorphisms, and no differences in longitudinal intestinal expression between proximal and distal sections. Overall, the results show that BCRP expression was constant along the entire gastrointestinal tract and did not vary widely among individuals, an observation consistent with previous reports on the intestinal expression of BCRP in man.[37] In contrast to the low inter-subject variability in BCRP protein expression, high inter-subject variability in sulfasalazine $AUC_{(0-\infty)}$ (20.5-fold) and C_{max} (17.2-fold) was observed, likely driven by individual differences in absorption and bacterial metabolism of sulfasalazine. Furthermore, Urquhart et al.[36] noted a 2.4-fold higher sulfasalazine $AUC_{(0-\infty)}$ in subjects with the 34GG/421CA genotype ($n = 5$) relative to subjects with the 34GG/421CC genotype ($n = 9$); this study did not enroll any 421AA subjects. Notably, a single subject with the 34GA/421CA genotype displayed a 4.8-fold higher $AUC_{(0-\infty)}$ compared to the 34GG/421CC group, suggesting that BCRP polymorphism may influence the plasma exposure of

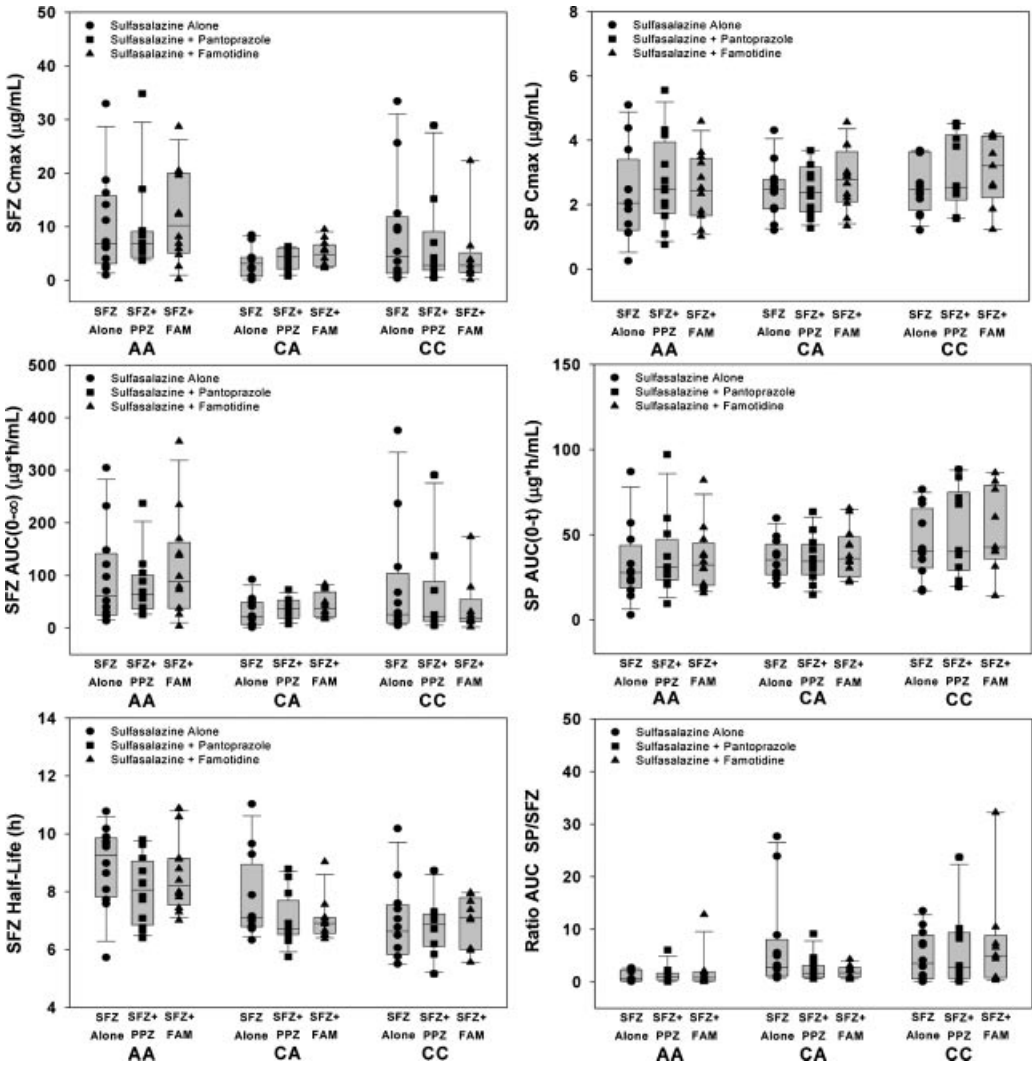

Figure 3. Comparison of individual sulfasalazine and sulfapyridine pharmacokinetic parameters among *ABCG2* 421 genotypic cohorts during Treatment Period 1 (Sulfasalazine Alone), Period 2 (Sulfasalazine + Pantoprazole (PPZ)), and Period 3 (Sulfasalazine + Famotidine (FAM)). Closed circles represent individual subject data. The boxes represent the median and 25th, 50th percentiles. Whiskers represent 5th and 95th percentiles.

sulfasalazine. However, in the present study in ethnic Chinese, the sulfasalazine $AUC_{(0-\infty)}$ observed in the seven 34GA/421CA subjects (39.6 ± 28.0 μg h/mL; mean ± SD) was well within the range of values observed in the other genotype groups. Indeed no consistent trends were noted across the G34A/C421A genotype groups, although subject numbers in some groups were limited and sulfasalazine variability was high.

The other report by Yamasaki et al.[30] investigated the impact of genetic polymorphisms of *ABCG2* and *NAT2* on the pharmacokinetics of sulfasalazine at a higher dose of 2000 mg in 37 healthy Japanese males prospectively screened for *ABCG2* 421CC ($n = 12$), CA ($n = 16$), and AA ($n = 9$) genotypes. Yamasaki et al.[30] used immediate release tablets and although the variability in sulfasalazine pharmacokinetic parameters was high, it was smaller than that reported by Urquhart et al.[36] or observed in the present study possibly due to the formulation or dose or a combination of both. Genetic analyses and population pharmacokinetic modeling revealed a strong relationship between *ABCG2* genotype and systemic exposure of sulfasalazine, with a 3.5-fold higher $AUC_{(0-48)}$ observed in the 421AA

Table 4. Sulfasalazine Plasma and Urine Pharmacokinetic Parameters in *ABCG2* 421CC, CA, and AA Genotype Cohorts and between Genotype Comparisons Following Oral Coadministration of 500 mg Sulfasalazine and 40 mg Pantoprazole

	ABCG2 421 Genotype			Ratio of Geometric LS Means [90% CI]	
	CC ($N = 10$)	CA ($N = 12$)	AA ($N = 12$)	CA vs. CC	AA vs. CC
$AUC_{(0-\infty)}$ (μg h/mL)	29.4 (12.1, 71.1) [190]	30.8 (20.3, 46.7) [73]	63.7 (42.3, 96.0) [72]	0.99 [0.40, 2.47]	2.05 [0.82, 5.12]
C_{max} (μg/mL)	3.60 (1.57, 8.28) [170]	3.31 (2.14, 5.13) [78]	7.43 (4.89, 11.3) [74]	0.86 [0.33, 2.24]	1.93 [0.75, 5.01]
T_{max} (h)	5.00 (4.00–8.00)	6.00 (4.00–8.00)	5.00 (3.00–8.00)	1.16 [0.94, 1.43]	1.08 [0.88, 1.33]
$T_{1/2}$ (h)	6.73 (6.09, 7.45) [14]	6.96 (6.43, 7.54) [13]	7.95 (7.25, 8.72) [15]	1.04 [0.91, 1.18]	1.18 [1.04, 1.35]
T_{lag} (h)	2.00 (0.00–3.00)	2.00 (0.75–4.00)	2.25 (1.00–5.00)	—	—
% Dose in urine	2.86 (1.29, 6.35) [157]	3.04 (1.94, 4.77) [81]	6.34 (4.04, 9.96) [81]	0.99 [0.41, 2.37]	2.06 [0.86, 4.94]

N, sample size in each genotype cohort.
Pharmacokinetic parameters shown as geometric mean (95% CI) and [CVb%], except T_{max} and T_{lag} which are presented as median (range).

Table 5. Sulfapyridine Plasma and Urine Pharmacokinetic Parameters in *ABCG2* 421CC, CA, and AA Genotype Cohorts and between Genotype Comparisons Following Oral Coadministration of 500 mg Sulfasalazine and 40 mg Pantoprazole

	ABCG2 421 Genotype			Ratio of Geometric LS Means [90% CI]	
	CC ($N = 10$)	CA ($N = 12$)	AA ($N = 12$)	CA vs. CC	AA vs. CC
$AUC_{(0-t)}$ (μg h/mL)	44.6 (30.4, 65.3) [58]	33.3 (25.8, 43.0) [42]	32.3 (22.3, 46.7) [63]	0.77 [0.49, 1.19]	0.74 [0.48, 1.16]
$AUC_{(0-\infty)}$ (μg h/mL)	60.7 (36.6, 101) [81]	41.6 (28.9, 59.8) [58] ($n = 11$)	37.0 (24.8, 55.3) [70]	0.73 [0.42, 1.26]	0.64 [0.37, 1.10]
C_{max} (μg/mL)	2.78 (2.09, 3.69) [41]	2.37 (1.91, 2.94) [35]	2.37 (1.64, 3.41) [62]	0.87 [0.58, 1.30]	0.87 [0.58, 1.30]
T_{max} (h)	12.0 (6.00–16.0)	12.0 (8.00–24.0)	10.0 (8.00–16.0)	1.10 [0.86, 1.41]	0.87 [0.68, 1.11]
$T_{1/2}$ (h)	10.8 (7.89, 14.9) [47]	8.12 (5.85, 11.3) [52] ($n = 11$)	7.48 (6.39, 8.75) [25]	0.85 [0.63, 1.15]	0.81 [0.60, 1.09]
T_{lag} (h)	3.50 (1.50–10.0)	4.00 (1.50–8.00)	4.00 (2.50–6.00)	—	—
% Dose in urine	5.74 (4.05, 8.13) [52]	3.86 (2.82, 5.28) [53]	4.23 (3.09, 5.79) [53]	0.68 [0.43, 1.08]	0.74 [0.47, 1.19]
$AUC(_{0-t})_{SP}/AUC_{(0-\infty)SFZ}$	2.85 (0.110–23.7)	1.65 (0.650–9.17)	0.920 (0.060–6.08)	0.78 [0.26, 2.39]	0.37 [0.12, 1.12]

N, sample size in each genotype cohort.
Pharmacokinetic parameters shown as geometric mean (95% CI) and [CVb%], except T_{max}, T_{lag} and AUC ratio which are presented as median (range).

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subjects compared to the 421CC subjects. Unlike our study, a clear gene-dose effect for sulfasalazine was observed with a ranking of AUC values being 421AA > 421CA > 421CC. Consistent with our study, Yamaski et al.[30] reported that sulfapyridine systemic exposures were lower in 421AA subjects compared to 421CC subjects and that *NAT2* genotype influenced sulfapyridine but not sulfasalazine systemic exposures.

These three studies provide insight into the utility of sulfasalazine as a clinical BCRP probe substrate. There were several similarities and differences among the reports. First, these studies clearly demonstrate that human pharmacokinetics of sulfasalazine are not as sensitive to BCRP efflux as that observed in mice. The influence of *ABCG2* C421A genotype on the plasma AUC of sulfasalazine in the three clinical studies, ranged from <2- to 4-fold over a dose range of 500–2000 mg (~7 to 30 mg/kg). In contrast, in Bcrp1 knockout mice, the $AUC_{(0-\infty)}$ of oral sulfasalazine at a dose of 20 mg/kg was more than 100-fold higher than in wild-type mice.[13] A number of mechanisms may contribute to the differences observed between humans and mice. For example, the 421A allele may retain partial BCRP activity in humans or sulfasalazine may be a substrate of other transporters that compensate for reduced BCRP activity in carriers of the C421A allele. To date, a null *ABCG2* allele has not been described in humans, nor has sulfasalazine been shown to be a substrate for other transporters. The species difference in the contribution of BCRP to sulfasalazine pharmacokinetics is important and is a strong reminder that one must be cautious when extrapolating findings from Bcrp1 knockout mice to humans.

A second similarity among the three studies was the observed high variability in the pharmacokinetics of sulfasalazine, with $AUC_{(0-\infty)}$ and C_{max} ranging approximately 20- to 80-fold, a finding consistent with previously reported literature[16,18] In contrast to the high inter-subject variability, the intra-subject variability is lower, as shown by the similar within subject pharmacokinetics across the three dosing treatments in our study (Fig. 1). This suggests that inter-individual differences in gastrointestinal physiology, absorption, and other factors, such as the extensive bacteria-mediated metabolism of sulfasalazine in the gut, are major contributors to the high between subject variability in sulfasalazine pharmacokinetics. Nonetheless, the high inter-subject variability observed in this and other studies makes sulfasalazine a less than optimal probe drug for assessing BCRP activity in clinical phenotyping or drug interaction studies.

There were several notable differences in the three sulfasalazine probe studies with regard to dose selection and formulation administered. The studies administered different doses: 500, 1000, and 2000 mg, which may contribute to differences in the magnitude of the effect of the *ABCG2* C421A polymorphism. Sulfasalazine doses of up to 4 g per day are used for the treatment of ulcerative colitis and 2 g per day for rheumatoid arthritis. However therapy is typically initiated at doses of 0.5–2 g per day to avoid gastrointestinal adverse effects (Azulfidine-EN and Salazopyrin-EN® Package Insert, Pfizer). In our study, the dose of 500 mg was selected to avoid any potential nausea/vomiting that could affect sulfasalazine absorption kinetics and to avoid potentially high sulfasalazine exposures when coadministered with a BCRP inhibitor. The doses in the other two studies were 2- and 4-fold higher than used in the present study. Interestingly, Yamasaki et al.[30] reported significant differences in the exposures of sulfasalazine for *ABCG2* 421A genotype when using the 2000 mg dose. Higher doses/intestinal concentrations may saturate the efflux of this poorly permeable compound by BCRP or some other unidentified transporter, resulting in dose-dependent effect. Future studies may be needed to identify the optimum dose if there is continued interest in sulfasalazine as a BCRP probe substrate.

Finally, different formulations were utilized across the studies. In the present study, the enteric coated formulation was used because of local availability. Urquhart et al.[36] dosed a 100 mL suspension prepared from crushed 500 mg immediate release tablets. The suspension yielded a 1.6-fold higher $AUC_{(0-\infty)}$ compared to the intact film coated 500 mg tablet; however, this difference in exposure was not statistically significant due to the large pharmacokinetic variability.[36] In contrast, immediate release tablets were used by Yamasaki et al.,[30] resulting in lower variability, an earlier T_{max} and presumably higher local concentrations of sulfasalazine in the intestinal tract, which may influence the interaction with the transporter. Although a cross-study comparison is challenging because of formulation, dose and population differences, the available data suggest that the immediate release formulation would be preferred and that use of the extemporaneously prepared suspension or enteric

coated tablet should be avoided. Unfortunately, the immediate release tablet is not available world-wide.

In conclusion, the results of this study suggest that the pharmacokinetic parameters of sulfasalazine from a 500 mg oral enteric coated tablet are not sufficiently sensitive to genetic variation or BCRP inhibition to use it as clinical probe for assessing BCRP activity. In addition, this study and two others indicate that BCRP-mediated efflux of sulfasalazine in humans is considerably less than that in mice, and that the majority of pharmacokinetic variability observed in humans after oral administration of sulfasalazine cannot be attributed to differences in BCRP-mediated efflux. An ideal probe drug would be specific for the transporter pathway of interest, sensitive to inhibitors, exhibit a wide safety margin, undergo minimal metabolism, be readily available in most countries, and useful in extrapolating drug interactions across multiple substrates and inhibitors. Sulfasalazine is less than ideal, as it has complex and extensive gut lumen metabolism coupled with high inter-subject variability and an apparent dose-dependence in sensitivity. Further research into BCRP probe substrates for use in clinical drug interaction studies is warranted.

SUPPORTING INFORMATION

Supporting information for this article is available (Table S1 Pharmacokinetic parameters of 5-aminosalicylic acid in the *ABCG2* 421CC, CA and AA genotype cohorts; Table S2 Pharmacokinetic parameters of sulfasalazine 500 mg when coadministered with 40 mg famotidine in the *ABCG2* 421CC, CA and AA genotype cohorts; Table S3 Pharmacokinetic parameters of sulfapyridine following sulfasalazine 500 mg when coadministered with 40 mg famotidine in the *ABCG2* 421CC, CA and AA genotype cohorts).

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