

Research Paper

Curcumin Inhibits the Activity of ABCG2/BCRP1, a Multidrug Resistance-Linked ABC Drug Transporter in Mice

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Purpose. To evaluate the *in vivo* efficacy of curcumin as an inhibitor of the multidrug-resistance-linked ATP Binding Cassette (ABC) drug transporter, ABCG2.

Methods. Photoaffinity labeling with [¹²⁵I]-iodoarylazidoprazosin was used to characterize the interaction of sulfasalazine, a substrate of the mouse ABCG2, with human ABCG2. In addition, the inhibitory effect of curcumin on ABCG2 was evaluated in brain capillaries from rats. Furthermore, the effect of curcumin on absorption of orally administered sulfasalazine in wild-type and *abcg2*^{-/-} mice was also determined.

Results. Sulfasalazine interacted at the drug-substrate site(s) of human ABCG2. Curcumin inhibited ABCG2 activity at nanomolar concentrations at the rat blood-brain barrier in the *ex vivo* assay. Based on studies in wild type and *abcg2*^{-/-} mice, we observed that oral curcumin increased *C*_{max} and relative bioavailability of sulfasalazine by selectively inhibiting ABCG2 function.

Conclusions. This study validates our previous *in vitro* results with human ABCG2 (Chearwae *et al.*, Mol. Cancer Ther. 5:1995–2006, 2006) and provides the first *in vivo* evidence for the inhibition by curcumin of ABCG2-mediated efflux of sulfasalazine in mice. Based on these studies, we propose that non-toxic concentrations of curcumin may be used to enhance drug exposure when the rate-limiting step of drug absorption and/or tissue distribution is impacted by ABCG2.

KEY WORDS: ABC transporter; ABCG2; curcumin; multidrug resistance; sulfasalazine.

INTRODUCTION

Curcumin (diferuloyl methane) is a naturally occurring polyphenol found in the rhizomes of *Curcuma longa* (turmeric). Curcumin is known for its antitumor, antioxidant, antiarthritic, anti-amyloid and anti-inflammatory properties (1–3). It has been found to suppress, retard, and even reverse cancer

development at each stage of the disease (4). The anticancer properties of curcumin have been primarily attributed to its activity to block nuclear factor-kappa B (NF-kappa B) which regulates inflammation, cell proliferation and apoptosis in normal cells (5–7).

ATP-binding cassette (ABC) transporters belong to a superfamily which transports a wide variety of substrates across extra- and intracellular membranes, including ions, sugars, metabolic products, lipids, sterols, toxins and drugs (8). Some of the ABC transporters play a crucial role in the development of multidrug resistance (MDR), as patients that are undergoing chemotherapy can eventually develop resistance not only to the anticancer drug they are taking but also to several other types of drugs (9). P-glycoprotein (P-gp), breast cancer resistance protein (BCRP or ABCG2), and multidrug resistance protein (MRP1) are the major ABC drug transporters that have been linked with MDR (9).

In addition to conferring MDR in tumor cells, in normal physiology ABC transporters limit the absorption of many drugs from the intestine, and pump drugs from the liver cells into the bile as a means of removing foreign substances from the body (10). In this regard, a large number of drugs are substrates that are themselves transported by ABC transporters or that affect the transport of other therapeutic drugs thereby altering the bioavailability of these drugs.

We have recently shown that curcumin inhibits the function of three major ABC drug transporters (P-gp, ABCG2 and MRP1) and curcumin I was most effective in

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ABBREVIATIONS: ABC, ATP binding cassette; AUC, area under concentration-time curve; BBB, blood-brain barrier; IAAP, iodoarylazidoprazosin; MDR, multidrug resistance; P-gp, P-glycoprotein; SASP, sulfasalazine.

interacting with ABCG2 (11–14). Based on our *in vitro* data, we proposed that curcumin can prevent chemotherapeutic drug resistance mediated by these transporters and in addition, can also improve the systemic availability of the cancer drugs that have limited intestinal absorption due to active efflux by these transporters. Thus, the aim of the present study was to demonstrate the ability of curcumin to inhibit one of the above ABC drug transporters, ABCG2 in an *in vivo* system. In our experimental approach, we used brain capillaries from rats to assess the inhibitory effect of curcumin on the efflux of BODIPY® FL prazosin *ex vivo* and also performed pharmacokinetic studies in mice using the ABCG2 specific substrate sulfasalazine (SASP) (15) to study the modulatory effect of curcumin on ABCG2 efflux activity *in vivo*. The data presented here strongly suggest that curcumin at clinically achievable non-toxic concentrations could be used to increase the bioavailability of ABCG2 substrates.

MATERIALS AND METHODS

Chemicals

Curcumin was from Sigma Aldrich (St. Louis, MO, USA). BODIPY® FL prazosin and FTC were from Molecular Probes (Eugene, OR, USA). Sulfasalazine (SASP; PNU-133) was from Pfizer Global research and Development (Kalamazoo, MI, USA). [¹²⁵I]-iodoarylazidoprazosin (IAAP; 2,200 Ci/mmol) was purchased from Perkin Elmer Life Sciences (Wellesley, MA, USA). All other chemicals were of highest grade and were purchased from Sigma (St. Louis, MO, USA).

Cells

Control MCF7 and MCF7FLV1000 (482R) cells over-expressing ABCG2 were cultured in RPMI with 10% FBS in the absence or presence of 1 µg/ml flavopiridol, respectively (16,17). These cell lines were provided by Dr. Susan Bates (NCI/NIH).

Isolation of Crude Membranes from ABCG2-expressing HEK 293 or MCF7 Cells

Crude membranes from MCF7 and MCF7FLV1000 cells were prepared as described elsewhere (11,18).

Animals

Male Sprague-Dawley rats (for brain capillary assays), male outbred mdr1aPGP (mdr1a^{-/-}) and CrI:CF-1 WT mice (for SASP pharmacokinetic studies) were purchased from Charles River Laboratories (Wilmington, MA, USA). Bcrp1^{-/-} (FVB.129S6-Abcg2tm1Ahs) and WT (FVB) mice (for SASP pharmacokinetic studies) were obtained from Taconic Laboratories (Germantown, NY, USA). Animals were housed and handled according to Pfizer Global Research & Development and University of Minnesota guidelines complying with the US Public Health Service Policy for the Care and Use of Laboratory Animals and were kept under controlled environmental conditions (23°C, 35% relative humidity, 12 h dark–light cycle) with free access to tap water

and standard rodent chow. Before using animals for experiments they were allowed to adapt to the animal facility for at least 5 days after transportation to the facility. Animal protocols were approved by the Institutional Animal Care and Use Committee of the University of Minnesota and were in accordance with AAALAC regulations and NIH animal guidelines.

Photoaffinity Labeling of ABCG2 with [¹²⁵I]-Iodoarylazidoprazosin (IAAP)

Crude membranes (1 mg protein/ml) from ABCG2-expressing MCF-7 FLV1000 cells were incubated with 0–100 µM SASP for 10 min at 21–23°C in 50 mM Tris-HCl, pH 7.5. 3–6 nM [¹²⁵I]-IAAP (2,200 Ci/mmol; Perkin Elmer Life Sciences, Wellesley, MA, USA) was added and incubated for an additional 5 min under subdued light. The samples were illuminated with a UV lamp (365 nm) for 10 min at room temperature. The labeled ABCG2 was immunoprecipitated as described previously (19). Samples were separated on a 7% Tris–acetate gel at constant voltage and gels were dried and exposed to X-ray film for 12–24 h at –80°C. The incorporation of [¹²⁵I]-IAAP into the ABCG2 or P-gp band was quantified using the STORM 860 phosphor imager system (Molecular Dynamics, Sunnyvale, CA, USA) and the software ImageQuaNT, as described (20).

Isolation of Brain Capillaries

Brain capillaries from rats were isolated as described previously (21–23). For each preparation, 10 rats were euthanized by CO₂ inhalation and decapitated. Brains were dissected and homogenized in PBS buffer (2.7 mM KCl, 1.46 mM KH₂PO₄, 136.9 mM NaCl, and 8.1 mM Na₂HPO₄ supplemented with 5 mM *d*-glucose and 1 mM sodium pyruvate, pH 7.4). After the addition of Ficoll (final concentration 15%), the homogenate was centrifuged at 5,800×*g* for 20 min at 4°C. The pellet was resuspended in PBS containing 1% BSA and passed over a glass bead column. Capillaries adhering to the glass beads were collected by gentle agitation in PBS (1% BSA), washed with PBS, and then used for transport experiments.

BODIPY® FL Prazosin Transport Assay in Rat Brain Capillaries

Transport assays using freshly isolated, functionally intact rat brain capillaries were done as reported previously (24,25) with minor modifications. Briefly, to measure ABCG2 transport function, isolated brain capillaries were incubated for 1 h at room temperature with 2 µM BODIPY® FL prazosin. For each treatment, images of 10 capillaries were acquired by confocal microscopy (Nikon C1 laser scanning confocal microscope unit, Nikon TE2000 inverted microscope, ×40 oil immersion objective, numerical aperture: 1.3, 488 nm line of a Spectra Physics argon laser (model 163C), 515/30 nm band pass filter; Nikon Instruments Inc., Melville, NY, USA). Images were analyzed by measuring fluorescence intensity of BODIPY® FL prazosin in the capillary lumen using Image J software (NIH, Bethesda, MD, USA) as described previously (25,26).

Statistical Analysis for BODIPY® FL Prazosin Transport Assays

Data are presented as mean ± SEM. Two-tailed unpaired Student's *t* test was used to evaluate differences between controls and treated groups; differences were considered to be statistically significant when $P < 0.05$. Sigmoidal dose-response curves and IC_{50} values were calculated using GraphPad Prism Software Version 4.01 (GraphPad Software, Inc., La Jolla, CA, USA).

Pharmacokinetic Experiments with Wild-type, *abcg2*^{-/-} and *abcb1a* (*mdr1a*^{-/-}) Mice

Curcumin or an equivalent volume of vehicle (in 0.5% methylcellulose in sterile phosphate buffer saline) was administered by oral gavage at a dose of 40 or 400 mg/kg followed by administration of SASP after 1 h to groups of mice at a dose of 20 mg/kg of body weight as previously described (15). Using the administration of SASP as time zero, mice were anesthetized with isoflurane at pre-determined time points, blood samples obtained by cardiac puncture and transferred to EDTA tubes. The samples were centrifuged immediately at 3,000×g for 15 min, and plasma was collected and stored at -80°C until the time of LC-MS/MS analysis.

Detection of Sulfasalazine Using LC-MS/MS and Pharmacokinetic Calculations

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, USA) as described earlier (15). Area under the concentration-time curve (AUC) from time zero to the last sampling time was calculated by the linear trapezoidal rule using non compartmental analysis of average plasma concentration in WinNonlin v5.1 (Pharsight Corporation, Mountain View, CA, USA 94041). Relative SASP exposures were estimated as the ratio of AUC in wild-type mice in the presence of curcumin *versus* wild-type mice in the absence of curcumin for each dose level examined in this study. Two Way ANOVA was used to evaluate the influence of curcumin on SASP C_{max} in wild type, *abcg2*^{-/-} and *abcb1a* (*mdr1a*^{-/-}) mice (GraphPad Software, La Jolla, CA, USA).

RESULTS

Curcumin Inhibits ABCG2 Activity at the Blood-Brain Barrier

In our previous study using cell lines overexpressing ABCG2, we showed that curcumin inhibited the activity of ABCG2 in *in vitro* assays (13). The objective of the present study was to demonstrate the inhibitory activity of curcumin on ABCG2 function under normal physiological conditions in an *in vivo* system. To demonstrate this in a model close to the *in vivo* situation, we used freshly isolated, functionally active rat brain capillaries. Isolated brain capillaries are an established *ex vivo* model for the blood-brain barrier, a tissue well-

known to express ABCG2 (25,27,28). At the blood-brain barrier, ABCG2 is localized to the luminal membrane, where it protects the brain by mediating substrate transport from brain-to-blood. Accordingly, in control capillaries that were incubated with fluorescent BODIPY® FL prazosin, an ABCG2 substrate, there was high fluorescence in the capillary lumen (Fig. 1a, left image). With our imaging conditions, no BODIPY® FL prazosin fluorescence was visible in the bath containing 2 μM BODIPY® FL prazosin. Fluorescence was low in the capillary endothelium and high in the capillary lumen indicating an active transport process. Quantitation of steady-state luminal BODIPY® FL prazosin accumulation showed that 5 μM fumitremorgin C (FTC), a known specific inhibitor of ABCG2 activity, reduced luminal fluorescence to approximately 50% of control capillaries (Fig. 1a, right panel and Fig. 1b). In capillaries treated with different concentrations of curcumin, luminal BODIPY® FL prazosin fluorescence was reduced in a dose-dependent manner (Fig. 1a,b). Inhibition of ABCG2 activity by curcumin was concentration-dependent with an IC_{50} value of 19.4 nM (Fig. 1c). Maximal inhibition of ABCG2 activity by 1–5 μM curcumin was comparable to the inhibition observed with 5 μM FTC (Fig. 1a,b). Thus, the above findings suggest that the *in vitro* inhibitory activity of curcumin on ABCG2 could also be observed with *in vivo* models where the transporter is present at normal physiological levels.

SASP, a Specific Substrate of Murine ABCG2, also Interacts with Human ABCG2

Due to high presystemic clearance, it is known that curcumin has a low bioavailability (29) and therefore it is possible that when given orally, curcumin may not be an effective systemic inhibitor of ABC transporters throughout the body. In this regards, we and other groups have shown that the absorption and elimination of the sulfa drug, SASP in the mouse is determined by ABCG2 as it is a substrate of this transporter (15,30). To further investigate the mechanism by which SASP may interact with human ABCG2, we monitored its effect on the photolabeling of ABCG2 with the known photoaffinity substrate, [¹²⁵I]-Iodoarylazidoprazosin (IAAP) (19). As shown in Fig. 2, SASP inhibited the binding of IAAP to ABCG2 in a concentration-dependent manner with an IC_{50} value of 1.02 μM. This confirmed that besides interacting with murine ABCG2, SASP also interacts with human ABCG2 at the substrate binding sites. In previous reports, we have shown that curcumin also interacts at the substrate binding pocket of ABCG2. Therefore it can probably be used as a competitive inhibitor to prevent SASP interactions with ABCG2, which was the basis for conducting the following *in vivo* experiments.

Curcumin Pretreatment Enhances Sulfasalazine Exposure in Mice

To investigate the interaction of curcumin with a known probe substrate whose systemic bioavailability is known to be greatly influenced by ABCG2 (15,30,31), we hypothesized that SASP absorption in the intestine could be enhanced through inhibition of the binding of SASP to mouse ABCG2. Briefly described, male FVB-WT mice were pretreated with a

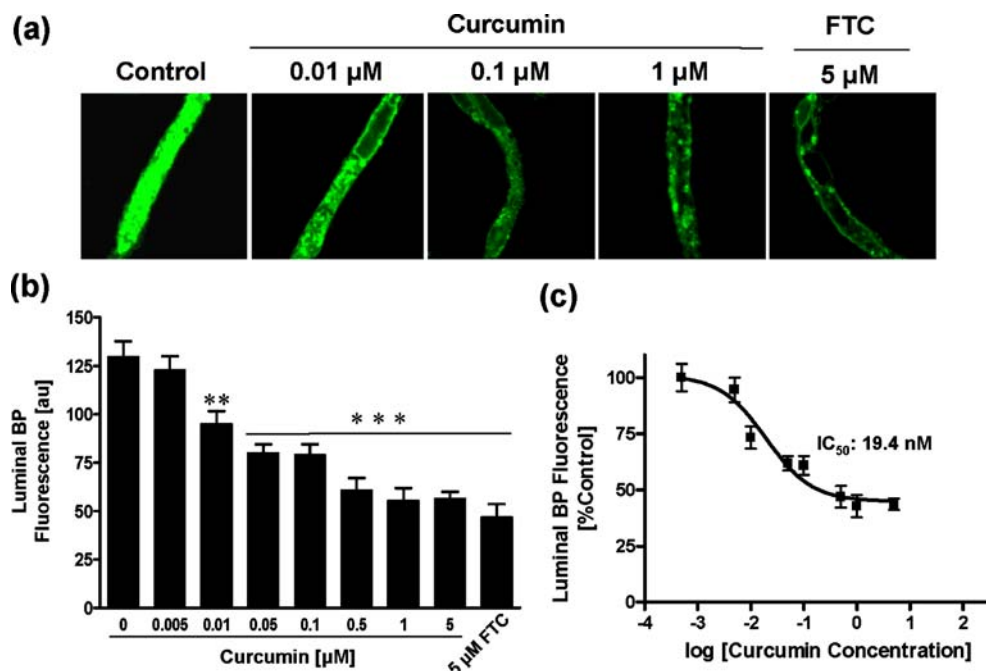


Fig. 1. Curcumin inhibits the activity of ABCG2 in rat brain capillaries. **a** Isolated, functionally intact rat brain capillaries were incubated with 2 μ M BODIPY® FL prazosin in the absence (control) or presence of varying concentrations of curcumin (middle three panels) and 5 μ M FTC at room temperature for 1 h and the images were acquired as described in “Materials and Methods”. Representative images from a single experiment from three individual experiments are shown here. **b** Luminal BODIPY® FL prazosin fluorescence in capillaries in the presence and absence of varying concentrations of curcumin was quantitated as described in “Materials and Methods” using Image J software and plotted as a function of curcumin concentrations. Statistical comparison: **, significantly lower than control capillaries, $P < 0.01$; ***, significantly lower than control capillaries, $P < 0.001$. **c** Non-linear regression curve; data are plotted on a logarithmic scale. Each data point represents the luminal BODIPY® FL prazosin fluorescence mean value from ten capillaries (pooled capillary tissue from ten rats of a single preparation); variability is given by SEM bars. Units are arbitrary fluorescence units (scale 0–255).

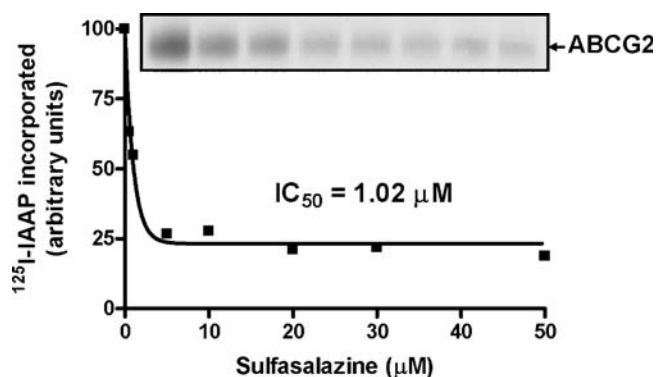


Fig. 2. Sulfasalazine inhibits the photolabeling of ABCG2 with [¹²⁵I]-IAAP binding. Crude membranes (500 μ g/ml) from MCF-7 FLV1000 cells were incubated with 0–50 μ M of SASP for 5 min at 21–23°C in 50 mM Tris-HCl, pH 7.5. 3–6 nM [¹²⁵I]-IAAP (2,200 Ci/mmol) was added and incubated for an additional 5 min under subdued light. The samples were then illuminated with a UV lamp (365 nm) for 10 min and were processed as described in “Materials and Methods”. A representative autoradiogram from one experiment is shown and similar results were obtained in two additional experiments. The arrow shows the position of the ABCG2 band. The incorporation of [¹²⁵I]-IAAP (from autoradiogram, Y-axis) into the ABCG2 (squares) band was quantified by estimating the radioactivity of this band plotted as a concentration of SASP using the software GRAPHPAD PRISM 2.0, as described previously (19).

single oral gavage of curcumin (40 or 400 mg/kg) 1 h prior to a single oral dose of SASP (20 mg/kg) whereby the plasma concentration of SASP was determined as a function of time in mice treated with SASP alone or a combination of curcumin and SASP. While no effect was observed when mice were pretreated with curcumin 40 mg/kg (data not shown), we report that the plasma concentration of SASP increased in the presence of curcumin (400 mg/kg) at all time points, suggesting that curcumin increased the relative bioavailability of SASP in the plasma probably by inhibiting intestinal ABCG2 (Fig. 3). It should be noted that at 24 h, SASP concentrations in control mice were below the limits of quantification (1 ng/mL), however, these samples were within the lower limit of detection (0.5 ng/mL). It is important to note that SASP plasma levels were still high in curcumin-treated mice after 24 h. Additional pharmacokinetic parameters were also moderate to significantly altered in the curcumin-treated mice; these results are summarized in Table 1. Co-administration of curcumin decreased the apparent oral clearance by ~10-fold (data not shown). Further, T_{max} (the time to achieve maximal plasma concentration) of SASP was increased from 0.5 to 2 h, while the apparent SASP plasma half-life was increased from 1.08 to 5.02 h (data not shown). The area under concentration-time curve (AUC) of SASP in the curcumin-treated mice increased to fivefold after 4 h and 13-fold after 24 h when compared to that of vehicle treated

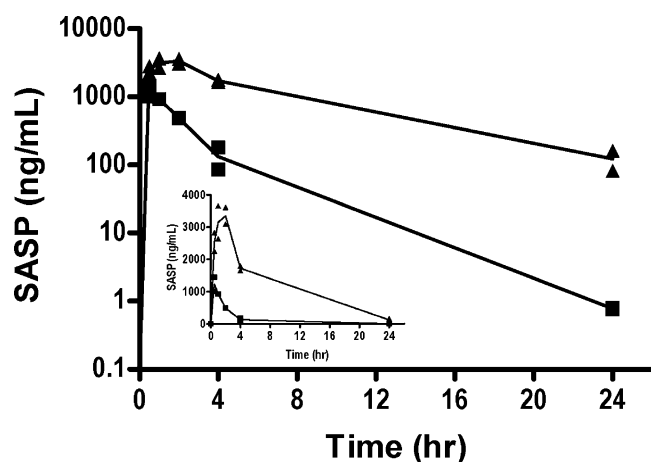


Fig. 3. Curcumin enhances plasma concentration of sulfasalazine in mice. Wild-type animals were pretreated with a single oral dose of curcumin (400 mg/kg) or vehicle for 1 h and all animals received a single oral dose of SASP (20 mg/kg). Line represents the average ($N=2$ /time point). Blood collected by cardiac puncture and the concentration of SASP was detected by LC-MS/MS as described in “Materials and Methods”. The main graph represents the log plasma concentration of SASP (Y-axis) with respect to time (X-axis) in vehicle treated (squares) or curcumin and SASP (triangles) treated mice. Inset shows the SASP concentrations on Y-axis in linear scale.

mice. In addition, 400 mg/kg curcumin treatment resulted in an increase in the maximal SASP plasma levels (C_{max}) of SASP from 1,230 ng/ml (vehicle) to 3,350 ng/ml (curcumin) in these mice. Taken together, the above results suggest that oral administration of curcumin enhanced the intestinal absorption of SASP in mice thereby increasing the apparent bioavailability of this ABCG2-specific substrate in these animals.

Curcumin Does not Affect the Plasma Concentration of Sulfasalazine in *abcg2* Knock-out Mice

To further rule out the possibility of the other major ABC drug transporter, P-gp's role in increasing the absorption of SASP, we did similar studies in wild-type, *abcb1a*^{-/-} (32) and *abcg2*^{-/-} mice (33). All mice received a single oral dose of vehicle or curcumin (400 mg/kg) followed by with a single oral dose of SASP (20 mg/kg) after 1 h. Ninety minutes post SASP dosing, the animals were sacrificed and plasma collected for the analysis of SASP. As shown in Fig. 4, the plasma concentrations of unchanged SASP in wild-type mice were increased from approximately 540 ng/ml (wild-type control) to approximately 2,070 ng/ml (wild-type mice treated with curcumin; Fig. 4). While *abcb1a*^{-/-} mice showed

similar levels of unchanged SASP with and without curcumin to wild-type mice, the *abcg2*^{-/-} mice had 12,350 ng/ml of SASP which was ~10–20 fold higher than the *abcb1a*^{-/-} and wild-type groups (Fig. 4). The presence of curcumin (400 ng/ml) did increase the plasma concentration of SASP three to fourfold in both wild-type and *abcb1a*^{-/-} mice, however, there were no significant effect on the levels of SASP in *abcg2*^{-/-} mice. Two-Way ANOVA using the Bonferroni post-test was used to determine the influence of curcumin inhibition (present/absent) with respect to the influence of genotype (WT, *abcb1a*, and *abcg2*) on SASP C_{max} (thus 3×two factors). Collectively, these data suggest that the observed effects of curcumin on the plasma levels of SASP were primarily mediated by inhibition of ABCG2 function, which is consistent with our current and published observation that SASP is a specific substrate of ABCG2 (Fig. 4 and (15,30,31)).

DISCUSSION

Oral administration of anticancer drugs is a convenient mode of drug delivery because of the simplicity in taking the drug, its potential use on an outpatient basis, and for development of chronic treatment schedules. However, the therapeutic use of orally administered drugs is frequently limited by poor and/or highly variable bioavailability. ABC drug transporters may restrict the oral bioavailability of their substrates by inhibiting the intestinal absorption of orally administered drugs (34). It has been shown by several groups that the oral bioavailability of certain anticancer drugs can be increased by inhibiting these ABC drug transporters in the intestine, which leads to increased drug response and thus, improved therapeutic outcome (35–37).

We showed previously that curcumin, a natural polyphenolic compound can be used to inhibit major ABC drug transporters such as P-gp, ABCG2 and MRP1 which play a major role in the development of MDR (11–13). Since curcumin is a relatively non-toxic nutraceutical with anti-cancer properties, we proposed that it could be used to increase the therapeutic efficacy of chemotherapy in MDR cancer cells by inhibiting the ABC drug transporters. In addition, it could also be used to increase the oral bioavailability of drugs that are substrates of ABC drug transporters.

Both P-gp and ABCG2 are known to be expressed on the apical membranes of brain capillary endothelial cells (38). We therefore used this system to demonstrate the *in vivo* efficacy of curcumin in inhibiting ABCG2 at the blood brain barrier of rats. Curcumin was effective in inhibiting the ABCG2-mediated efflux of BODIPY® FL prazosin with an IC_{50} of 0.019 μ M. The IC_{50} value of curcumin in these *ex vivo*

Table I. Effect of Curcumin on Sulfasalazine, Maximal Plasma Concentration (C_{max}), T_{max} and Area Under Concentration-time Curve (AUC) in Mice

Curcumin PO dose (mg/kg)	C_{max} (ng/ml)		T_{max} (h)		Interval (h)	AUC (ng h/ml)		Relative exposure AUC_{C+SASP}/AUC_{SASP}
	SASP	C + SASP	SASP	C + SASP		SASP	C + SASP	
400	1,230	3,350	0.5	2	0–4	2,170	10,380	5.0
					0–24	2,170 ^a	28,802	13 ^a

^a $AUC_{0-24 C+SASP}/AUC_{0-24 SASP}$

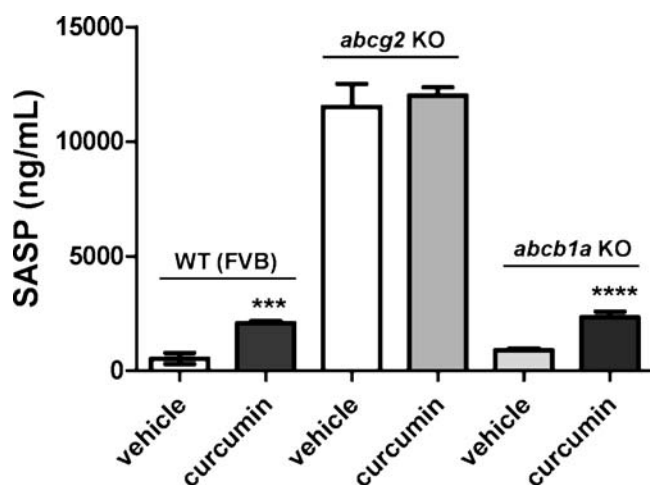


Fig. 4. Effect of curcumin on the plasma concentration of sulfasalazine in *abcb1a*, and *abcg2* knock-out mice. All animals were treated with a single oral dose of curcumin (400 mg/kg) for 1 h followed by a single oral dose of sulfasalazine (20 mg/kg). At a pre-determined time point (90 min), mice were anesthetized with isoflurane, and blood samples were obtained by cardiac puncture and transferred to heparinized tubes. The samples were centrifuged immediately at 3,000×g for 15 min, and plasma was collected and stored at -80°C until the time of LC-MS/MS analysis. $N=5$ /treatment. Data reported as mean±SD. Two way ANOVA was used to compare the effect of curcumin *versus* vehicle on SASP absorption in wild type (FVB), *abcg2*^{-/-} (*abcg2* KO), and *abcb1a*^{-/-} (*mdr1a* KO) mice. Using Bonferroni's post-hoc test, the effect of curcumin pretreatment significantly increased SASP concentration in the wild-type ($P<0.001$) and *mdr1a* (*abcb1a*^{-/-}; $P<0.001$) mice. However, there was no significant increase in SASP exposure in *abcg2*^{-/-} mice after curcumin pretreatment ($P>0.05$). As previously reported, *abcg2* genotype significantly influences SASP Cmax ($P<0.0001$) (15).

assays for inhibiting ABCG2 activity is different from our previous results where we reported that curcumin inhibited ABCG2 activity in *in vitro* cultured cell lines with an IC_{50} of 1–2 μ M (13). These differences in the IC_{50} values could be due to differences in the ABCG2 expression levels and altered substrate specificities between species. In addition, the brain capillaries used in this study have normal physiological levels of rat ABCG2, while in our previous study, we used either drug-selected or transfected cell lines that over-express human ABCG2 at high levels. Although FTC inhibited 50% of the efflux of BODIPY®-prazosin in the brain capillaries, we did observe a residual fluorescence in these cells. This may be due to passive diffusion, non specific binding, presence of secondary transporters responsible for the uptake of the fluorescent compound or other active ABC drug transporters at the blood brain barrier which were not inhibited by FTC (24,26).

In this study, we also assessed the effect of curcumin on the oral absorption of the ABCG2-specific substrate SASP in mice. Our data suggest that pretreatment of mice with a single oral dose of curcumin resulted in a 2-fold higher plasma level of unchanged SASP in both wild-type and *abcb1*^{-/-} mice. Our observation is also in agreement with previously published results indicating a minor role for P-gp in SASP availability after oral intake (15). Moreover, pretreatment of *abcg2*^{-/-} mice with curcumin resulted in similar plasma

concentrations of SASP in both treatment groups, also supporting a lesser role of P-gp in SASP oral absorption. Taken together, our data strongly support an important role of *abcg2* in sulfasalazine oral absorption. We have previously shown that *abcg2* not only limits the oral absorption of SASP in mice (15) but also influences the distribution and elimination processes of this drug in the same animals. Therefore, it is not surprising that our current data shows a decrease in oral clearance and volume of distribution and an increase in the half-life of SASP in the wild-type mice treated with curcumin.

This indicates that our *in vitro* results can be translated to *in vivo* systems in which, curcumin enhances the oral bioavailability of substrates that otherwise are hampered by active efflux by ABC drug transporters in the intestine. Although clinical trials in humans indicated that the systemic bioavailability of orally administered curcumin is relatively low (29), but it has been shown that curcumin formulation with phosphatidylcholine is fivefold more bioavailable than normal curcumin (39). In addition, it has recently been shown that oral administration of 8 g curcumin daily up to 18 months is well tolerated and despite its limited bioavailability, also inhibited progression of pancreatic cancer (40). Several other studies have shown that curcumin bioavailability can be enhanced considerably by using different approaches such as using adjuvants, making nanoparticles, liposomal formulation or making derivatives (reviewed in (41)).

Although curcumin is known to possess anticancer properties by virtue of its ability to inhibit key targets in cancer cells, this study provides additional *in vivo* evidence supporting its effectiveness in inhibiting ABC drug transporters. Both experiments using brain capillaries *ex vivo* assays and studies with mice *in vivo* showed convincingly that curcumin can enhance the drug (SASP) accumulation by inhibiting ABCG2 thereby increasing its intracellular concentrations.

Taken together, these non-clinical observations may be potentially clinically translatable with respect to curcumin administered orally in combination with other anticancer agents in cancer chemotherapy and hence could enhance the effectiveness of the drug treatment. The concept of using combination therapy to treat drug-resistant cancer or to improve the oral availability of certain drugs is not new and several inhibitors have been generated in the last 10 years (reviewed in (42)). However, the usefulness of such inhibitors to overcome MDR is still controversial and perhaps limited by the inhibition of ABCB1 in a non-tumor selective manner. It is noteworthy to recognize that a large number of these inhibitors are natural products or their purified constituents derived from varying sources ranging from fungi to plants and vegetables (42). Therefore, these natural inhibitors are expected to be much less toxic to the host than synthetic compounds. The advantage of using curcuminoids as inhibitors lies in their relatively large therapeutic-index compared to most of the other third generation MDR inhibitors. For example, Ko143 and GF120918, which are well characterized and potent inhibitors of ABCG2 and ABCB1, have an IC_{50} value of 19 and 18 μ M for MCF-7 cells (43), respectively, while curcuminoids have IC_{50} values in the range of 25–50 μ M for the same cells (13). Therefore, curcumin or its formulation with improved bioavailability such as curcumin

encapsulated into liposomes may represent an ideal lead compound that could be developed as a broad spectrum inhibitor of ABC drug transporters.

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