Small intestinal efflux mediated by MRP2 and BCRP shifts sulfasalazine intestinal permeability from high to low, enabling its colonic targeting

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Dahan A, Amidon GL. Small intestinal efflux mediated by MRP2 and BCRP shifts sulfasalazine intestinal permeability from high to low, enabling its colonic targeting. Am J Physiol Gastrointest Liver Physiol 297: G371–G377, 2009. First published June 18, 2009; doi:10.1152/ajpgi.00102.2009.—Sulfasalazine is characterized by low intestinal absorption, which essentially enables its colonic targeting and therapeutic action. The mechanisms behind this low absorption have not yet been elucidated. The purpose of this study was to investigate the role of efflux transporters in the intestinal absorption of sulfasalazine as a potential mechanism for its low small-intestinal absorption and colonic targeting following oral administration. The effects of P-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and breast cancer resistance protein (BCRP) inhibitors on sulfasalazine bidirectional permeability were studied across Caco-2 cell monolayers, including dose-response analysis. Sulfasalazine in vivo permeability was then investigated in the rat jejunum by single-pass perfusion, in the presence vs. absence of inhibitors. Sulfasalazine exhibited 19-fold higher basolateral-to-apical (BL-AP) than apical-to-basolateral (AP-BL) Caco-2 permeability, indicative of net mucosal secretion. MRP2 inhibitors (MK-571 and indomethacin) and BCRP inhibitors [fumitremorgin C (FTC) and pantoprazole] significantly increased AP-BL and decreased BL-AP sulfasalazine Caco-2 transport in a concentration-dependent manner. No effect was observed with the P-gp inhibitors verapamil and quindine. The IC50 values of the specific MRP2 and BCRP inhibitors MK-571 and FTC on sulfasalazine secretion were 21.5 and 2.0 μM, respectively. Simultaneous inhibition of MRP2 and BCRP completely abolished sulfasalazine Caco-2 efflux. Without inhibitors, sulfasalazine displayed low (vs. metoprolol) in vivo intestinal permeability in the rat model. MK-571 or FTC significantly increased sulfasalazine permeability, bringing it to the low-high permeability boundary. With both MK-571 and FTC present, sulfasalazine displayed high permeability. In conclusion, efflux transport mediated by MRP2 and BCRP, but not P-gp, shifts sulfasalazine permeability from high to low, thereby enabling its colonic targeting and therapeutic action. To our knowledge, this is the first demonstration of intestinal efflux acting in favor of oral drug delivery.

efflux transporters; intestinal absorption; passive/active gastrointestinal wall transport

ATP-DEPENDENT EFFLUX TRANSPORTERS, such as P-glycoprotein (P-gp; ABCB1), multidrug resistance-associated protein 2 (MRP2; ABCB2), and breast cancer resistance protein (BCRP; ABCG2), have been shown to play a significant role in drug absorption, distribution, and clearance processes, as well as in drug-drug and drug-food interactions (2, 13, 21, 23). These transporters present on many biological membranes, including the villus tip of the apical brush-border membrane of gut enterocytes, and actively cause efflux of drugs from gut epithelial cells back into the intestinal lumen. Attributable to overlapping substrate specificities, multiple efflux pumps might be involved in limiting membrane permeability of a given drug molecule. Drug substances that have been shown to be cosubstrates for several efflux pumps include atorvastatin (14), rosuvastatin (11), saquinavir (25), and fexofenadine (18). It is necessary to determine the contribution of each transporter to the overall efflux process because such information enables the prediction of changes in membrane permeability when the functions of transporters are altered, e.g., by genetic polymorphism, disease state, or drug-drug interactions.

Sulfasalazine (Fig. 1) has long been the mainstay of treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease. Following oral administration, sulfasalazine is characterized by low intestinal absorption (2% to maximally 13%) (12, 22) that essentially enables its colonic targeting and therapeutic action; upon arrival to the colon, the azobridge is cleaved by colonic bacterial azo reductases, to form sulfapyridine and 5-aminosalicylic acid (20). The reasons for sulfasalazine low oral bioavailability were investigated. It was attributed initially to low solubility and permeability characteristics (27); however, sulfasalazine has calculated log P (CLog P) value of 3.88, indicative of high permeability compound (9). The involvement of various efflux transporters was also suggested (15, 19). Recently, Zaher et al. (28) reported that BCRP plays an important role in sulfasalazine oral bioavailability in mice. However, the potential role of other efflux transporters in sulfasalazine intestinal absorption has not yet been elucidated. Moreover, the relative contribution of the efflux transport to the overall low intestinal absorption of sulfasalazine has not yet been addressed.

The purpose of this study was to investigate the role of efflux transporters in the intestinal absorption of sulfasalazine, as a potential mechanism for its low small-intestinal absorption, and colonic targeting, following oral administration. The effects of various P-gp, MRP2, and BCRP inhibitors on the bidirectional transepithelial permeability of sulfasalazine were studied across Caco-2 cell monolayers, inhibiting one transporter at a time vs. multiple transporters simultaneously. The effective permeability of sulfasalazine was then investigated by the in situ single-pass intestinal perfusion model, in the presence vs. absence of specific MRP2 (MK571) and BCRP (FTC) inhibitors, again one at a time vs. simultaneous inhibition. Overall, this setup allowed us to confirm the role of the different efflux pumps in sulfasalazine intestinal permeability, contributing to better understanding of the mechanisms behind its low oral bioavailability and enabling the prediction of changes attributable to altered transporter function.

MATERIALS AND METHODS

Materials. Sulfasalazine, metoprolol, phenol red, verapamil, quindine, indomethacin, fumitremorgin C (FTC), Lucifer yellow, M6S buffer, glucose, CaCl2, MgCl2, and trifluoroacetic acid were purchased...
from Sigma Chemical (St. Louis, MO). Pantoprazole, potassium chloride, and NaCl were obtained from Fisher Scientific (Pittsburgh, PA). MK-571 was purchased from Alexis Biochemicals (Lausen, Switzerland). Acetone-nitrile and water (Acros Organics, Geel, Belgium) were HPLC grade. Physiological saline solution was purchased from Hospira (Lake Forest, IL). All other chemicals were of analytical reagent grade.

Cell culture. Caco-2 cells (passages 18-26) from American Type Culture Collection (Rockville, MD) were routinely maintained in DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS, 1% nonessential amino acids, 1 mM sodium pyruvate, and 1% l-glutamine. Cells were grown in an atmosphere of 5% CO2 and 90% relative humidity at 37°C. The culture medium was routinely replaced by fresh medium every three days. Cells were passaged upon reaching ~80% confluence.

Caco-2 permeability studies. Transepithelial transport studies were performed in a method described previously (4, 8). Briefly, 5 × 10⁴ cells/cm² were seeded onto collagen-coated membranes (12-well Transwell plate, 0.4-μm pore size, 12-mm diameter; Corning Costar, Cambridge, MA) and were allowed to grow for 21 days. Mannitol and Lucifer yellow permeabilities were assayed for each batch of Caco-2 monolayers (n = 3), and transepithelial electrical resistance (TEER) measurements were performed on all monolayers (Millicell-ERS epithelial Voltohmmeter; Millipore, Bedford, MA). Monolayers with apparent mannitol and Lucifer yellow permeability <3 × 10⁻⁷ cm/s and TEER values >300 Ωcm² were used for all studies. On the experiment day, the monolayers were rinsed and incubated for 20 min with a blank transport buffer. The transport buffer contained 1 mM CaCl₂, 0.5 mM MgCl₂·6H₂O, 145 mM NaCl, 3 mM KCl, 1 mM NaH₂PO₄, 5 mM d-glucose, and 5 mM MES. Similar pH was used in both apical (AP) and basolateral (BL) sides (pH 6.5) to maintain constant degree of ionization in both AP-BL and BL-AP direction experiments and to avoid possible influence of this factor on the permeability across the cells. Following the 20-min incubation, the drug-free transport buffer was removed from the donor side (AP in the AP-BL-direction studies or BL in the BL-AP-direction studies) and replaced by sulfasalazine uptake buffer solution (pH 6.5), with or without inhibitors. Throughout the experiment, the transport plates were kept in a shaking incubator (50 revolution/min) at 37°C. Samples were taken from the receiver side at various time points up to 120 min (100 μl from BL side or 70 μl from AP side), and similar volumes of blank buffer were added following each sample withdrawal. At the last time point (120 min), a sample was taken from the donor side as well to confirm mass balance. Samples were immediately assayed for drug content. All Caco-2 monolayers were checked for confluence by measuring the TEER before and after the transport study (TEER values remained steady throughout the experiment).

Inhibition experiments. The concentration-dependent effects of the P-gp inhibitors verapamil (75 and 150 μM) and quinidine (50 and 100 μM), the MR2 inhibitors MK571 and indomethacin (20 and 100 μM), and the BCRP inhibitors FTC (10 and 20 μM) and pantoprazole (20 and 100 μM) on the bidirectional transport of sulfasalazine (0.1 mM) across Caco-2 cell monolayers were examined. The inhibitors were added only to the donor side of the Caco-2 cell system, i.e., to the AP side in the AP-BL experiments, or to the BL side in the BL-AP experiments. The inhibitors were added only at the start point of the experiment, together with the sulfasalazine, i.e., with no preexperimental incubation. The results were evaluated compared with sulfasalazine transport in the absence of inhibitors. The effect of the MR2 and BCRP inhibitor combination MK571 (100 μM) and FTC (20 μM) on sulfasalazine bidirectional transport was then investigated. These inhibitor concentrations were identified from their concentration-dependent effects on sulfasalazine secretion, as described below.

**Determination of the IC₅₀ of MK-571 and FTC on sulfasalazine transport.** The concentration-dependent effects of the specific MR2 inhibitor MK-571 (0.1–100 μM) and the specific BCRP inhibitor FTC (0.1–40 μM) on the secretory (BL-AP) direction transport of 0.1 mM sulfasalazine were investigated. The IC₅₀ of the tested compounds on sulfasalazine transport was then determined from the dose-response curve with the use of the percentage inhibited for each inhibitor concentration. The percentage inhibited in each concentration was calculated by dividing the F_sps by the control apparent permeability value. Michaelis-Menten parameters and IC₅₀ value were then determined using nonlinear regression according to the Hill equation with GraphPad Prism 4.01 (GraphPad Software, San Diego, CA). This set of experiments allowed us to identify the inhibitor concentrations to be used in the following rat studies.

**Single-pass intestinal perfusion studies in rats.** All animal experiments were conducted using protocols approved by the University Committee of Use and Care of Animals (UCUCA), University of Michigan, and the animals were housed and handled according to the University of Michigan Unit for Laboratory Animal Medicine (ULAM) guidelines. Male albino Wistar rats (Charles River, IN) weighing 250–280 g were used for all perfusion studies. Before each experiment, rats were fasted overnight (12–18 h) with free access to water. Animals were randomly assigned to the different experimental groups.

The procedure for the in situ single-pass intestinal perfusion followed previously published reports (7, 10). Briefly, rats were anesthetized with an intramuscular injection of 1 ml/kg ketamine-xylazine solution (9:1%, respectively) and placed on a heated surface maintained at 37°C (Harvard Apparatus, Holliston, MA). The abdomen was opened by a midline incision of 3–4 cm. A jejunal segment (5 cm average distance of the inlet from the ligament of Treitz) of ~10 cm was carefully exposed and cannulated on two ends with flexible PVC tubing (2.29 mm ID, inlet tube 40 cm, outlet tube 20 cm; Fisher Scientific, Pittsburgh, PA). Care was taken to avoid disturbance of the circulatory system, and the exposed segment was kept moist with 37°C normal saline solution. All solutions were incubated in a 37°C water bath. The isolated segment was rinsed with blank perfusion buffer to clean out any residual debris.

At the start of the study, perfusion solution containing sulfasalazine (0.1 μM), 10 mM MES buffer, pH 6.5, 135 mM NaCl, 5 mM KCl, and 0.1 mg/ml phenol red with an osmolarity of 290 mOsm/l, with or without the different inhibitors (20 μM FTC and 100 μM MK-571), was perfused through the intestinal segment (Watson Marlow Pumps 323S; Watson-Marlow Bredel, Wilmington, MA), at a flow rate of 0.2 ml/min. Phenol red was added to the perfusion buffer as a nonabsorbable marker for measuring water flux. Metoprolol was coperfused with the sulfasalazine as well, as a compound with known permeability that serves as a marker for the integrity of the experiment and as a reference standard for permeability in close proximity to the low/high permeability class boundary (5, 10). The perfusion buffer was first perfused for 1 h to assure steady-state conditions (as also assessed by the inlet-over-outlet concentration ratio of phenol red, which approaches 1 at steady state). Following reaching steady state, samples were taken in 10-min intervals for 1 h. All samples including perfusion samples at different time points, original drug solution, and inlet solution taken at the exit of the syringe were immediately
assayed by HPLC. Following the termination of the experiment, the length of each perfused intestinal segment was accurately measured.

**Data analysis.** Permeability coefficient ($P_{app}$) across Caco-2 cell monolayers was calculated from the linear plot of drug accumulated in the receiver side vs. time, using the following equation

$$P_{app} = \frac{1}{C_0 A} \times \frac{dQ}{dt}$$

where $dQ/dt$ is the steady-state appearance rate of the drug on the receiver side, $C_0$ is the initial concentration of the drug in the donor side, and $A$ is the monolayer growth surface area (1.12 cm$^2$). Linear regression was carried out to obtain the steady-state appearance rate of the drug on the receiver side ($r^2 > 0.99$ in all experimental groups).

The efflux ratio (ER) (i.e., the net efflux of sulfasalazine) was determined by calculating the ratio of $P_{app}$ in the secretory (BL-AP) direction divided by the absorptive (AP-BL) direction $P_{app}$, according to the following equation

$$ER = \frac{P_{app, BL-AP}}{P_{app, AP-BL}}$$

The effective permeability ($P_{eff}$) through the rat gut wall in the single-pass intestinal perfusion studies was determined according to the following equation

$$P_{eff}(cm/s) = \frac{-Q \ln(C_{out}/C_{in})}{2\pi rl}$$

where $Q$ is the perfusion buffer flow rate, $C_{out}/C_{in}$ is the ratio of the outlet/inlet sulfasalazine concentration that has been adjusted for water transport, $r$ is the radius of the intestinal segment (set to 0.2 cm), and $l$ is the length of the perfused intestinal segment.

The net water flux in the single-pass intestinal perfusion studies was determined by measurement of phenol red, a nonabsorbed, nonmetabolized marker. The phenol red (0.1 mg/ml) was included in the perfusion buffer and coperfused with the tested drugs. The measured $C_{out}/C_{in}$ ratio was corrected for water transport according to the following equation

$$\frac{C'_{out}}{C'_{in}} = \frac{C_{out}}{C_{in}} \times \frac{C_{in, phenol red}}{C_{out, phenol red}}$$

where $C_{in, phenol red}$ is equal to the concentration of phenol red in the inlet sample, and $C_{out, phenol red}$ is equal to the concentration of phenol red in the outlet sample.

**Analytical methods.** The amount of sulfasalazine in the Caco-2 medium and the simultaneous analysis of sulfasalazine, metoprolol, and phenol red in the rat perfusion buffer were assayed using a HPLC system (Waters 2695 Separation Module; Waters, Milford, MA) with a photodiode array UV detector (Waters 2996). Samples were filtered (Unifilter 96-well microplate 0.45 μm filters; Whatman, Florham Park, NJ), and Caco-2 medium aliquots of 50 μl or rat perfusion aliquots of 10 μl were injected into the HPLC system. The HPLC conditions were as follows: XTerra, RP$_{18}$, 3.5 μm, 4.6 × 100 mm column (Waters); a gradient mobile phase, going from 75:25 to 40:60% vol/vol aqueous/organic phase, respectively, over 18 min; the aqueous phase was 0.1% trifluoroacetic acid in water, and the organic phase was 0.1% trifluoroacetic acid in acetonitrile; flow at a rate of 1 ml/min in room temperature. The detection wavelengths were 275, 266, and 350 nm, and the retention times were 5.6, 7.7, and 15.6 min for metoprolol, phenol red, and sulfasalazine, respectively. Separate standard curves were used for each experiment ($r^2 > 0.99$).

**Statistical analysis.** All Caco-2 experiments were performed in triplicate (unless stated otherwise), and all animal experiments were replicate (unless stated otherwise), and all animal experiments were $n = 4$. The data are presented as means ± SD. To determine statistically significant differences among the experimental groups, the nonparametric Kruskal-Wallis test was used for multiple comparisons, and the two-tailed nonparametric Mann-Whitney U-test for two-group comparison when appropriate. A $P$ value of <0.05 was termed significant.

**RESULTS**

**The effect of different efflux transporters on sulfasalazine transepithelial permeability across Caco-2 cell monolayers.** The flux of sulfasalazine across Caco-2 monolayers in the AP-BL and BL-AP directions, in the presence vs. absence of different concentrations of MRP2 or BCRP inhibitors, is pre-
sented in Figs. 2 and 3, respectively. All MRP2 and BCRP inhibitors significantly increased AP-BL and decreased BL-AP direction transport in a concentration-dependent manner. Among these inhibitors, the specific MRP2 inhibitor MK-571 and the specific BCRP inhibitor FTC showed the most potent effect; however, neither of these inhibitors could demonstrate an efflux ratio of 1, i.e., complete inhibition of the drug efflux.

The effects of the P-gp inhibitors verapamil and quinidine on sulfasalazine bidirectional transport across Caco-2 cell monolayers are presented in Fig. 4. It can be seen that sulfasalazine transepithelial permeability was not affected by the inhibition of P-gp. The Caco-2 expression of P-gp, MRP2, and BCRP was validated using Western blot analysis (6), confirming that these efflux transporters were indeed present in the cell culture experiments in the protein level.

The dose-response curves for the inhibition of sulfasalazine (0.1 mM) mucosal secretion in the secretory (BL-AP) direction by the specific MRP2 and BCRP inhibitors MK-571 and FTC are shown in Fig. 5. Both compounds displayed concentration-dependent inhibition on sulfasalazine mucosal secretion with
high efficacy, reducing sulfasalazine BL-AP secretion to 37.5% and 42.2%, respectively, compared with the control. FTC was found to be highly potent with an IC50 value of 2.0 μM, whereas MK-571 demonstrated IC50 values of 21.5 μM. Higher MK-571 concentrations could not be evaluated because of solubility limitations, and, hence, values for this inhibitor represent best-fit assessment. It can be seen that sulfasalazine BL-AP transport in the presence of 20 μM FTC was in the lower plateau region, and 100 μM MK-571 approached this region, indicating maximal inhibition of BCRP- and MRP2-mediated sulfasalazine secretion. Hence, these concentrations were used in the following studies.

The effect of simultaneous MRP2 and BCRP inhibition by MK-571 and FTC combination, on the bidirectional Caco-2 transport of sulfasalazine is presented in Fig. 6, and the resulted efflux ratios of sulfasalazine from the different inhibition experiments are summarized in Fig. 7. It can be seen that the MK-571 and FTC combination was able to completely abolish sulfasalazine efflux transport, as symmetrical transport, i.e., AP-BL=BL-AP, was obtained.

The effect of MRP2 and BCRP on sulfasalazine intestinal permeability across the rat small intestine. Sulfasalazine permeability coefficients (Peff) obtained following in situ perfusion to the rat jejunum, in the presence vs. absence of the specific MRP2 inhibitor MK-571 or the specific BCRP inhibitor FTC, as well as the simultaneous combination of the two, are presented in Fig. 8. Without inhibitors, sulfasalazine displayed low (in comparison to metoprolol) permeability in the rat small intestine. The presence of either MK-571 or FTC significantly increased sulfasalazine permeability, bringing it to the level of metoprolol, i.e., the low-high permeability class boundary. With both MK-571 and FTC present, sulfasalazine was significantly higher than metoprolol, i.e., a complete shift from low to high permeability compound was observed.

DISCUSSION

Although intestinal efflux transport processes present a significant barrier toward drug absorption following oral administration and conventionally the challenge is to overcome it, the data presented in this paper reveal an opposite scenario. Sulfasalazine low absorption from the small intestine essentially enables its colonic targeting and therapeutic action. We have clearly shown that sulfasalazine susceptibility to intestinal efflux shifts the drug from high- to low-permeability compound, resulting in its low intestinal absorption, thereby enabling its colonic targeting and therapeutic action. This can also be construed as a novel method to achieve colonic targeting. To our knowledge, this is the first demonstration of intestinal efflux acting in favor of oral drug delivery.

Sulfasalazine BCRP-mediated efflux has been demonstrated before (26, 28); however, even when BCRP was completely inhibited, an efflux ratio of 1 could not be obtained, indicating the involvement of multiple systems in sulfasalazine efflux. Moreover, the relative contribution of the efflux transport to the overall low intestinal absorption of sulfasalazine has not been previously addressed. Hence, we investigated the role of the main intestinal efflux transporters, P-gp, MRP2, and BCRP,
in sulfasalazine bidirectional permeability. The specific MRP2 inhibitor MK-571 and the nonspecific MRP2 inhibitor indomethacin resulted in a strong concentration-dependent increase in sulfasalazine AP-BL transport, accompanied by a decrease in BL-AP transport, illustrating that sulfasalazine is susceptible to MRP2-mediated efflux transport. Similar results were obtained with the specific BCRP inhibitors FTC and pantoprazole, whereas P-gp inhibition had no effect on sulfasalazine bidirectional transport. Overall, it was revealed that combined effect of MRP2 and BCRP, but not P-gp, mediates the trans-epithelial efflux transport of sulfasalazine. This was made more evident when the MRP2 and BCRP inhibitors were used in combination (Fig. 6); the simultaneous presence of MK-571 and FTC completely abolished sulfasalazine efflux transport. Hence, a comprehensive characterization of sulfasalazine transepithelial efflux process was achieved.

According to the Biopharmaceutics Classification System (BCS) principles, all compounds are classified into one of four biopharmaceutical classes according to their water solubility and membrane permeability characteristics (1, 3, 17). In a provisional BCS classification, sulfasalazine was classified as a Class II compound, i.e., low-solubility high-permeability compound, on the basis of its CLog P value (3.88) (9). The data presented in this paper show that, in fact, sulfasalazine is a BCS Class IV compound, i.e., low-solubility low-permeability compound (Fig. 8). Indeed, under conditions in which the relevant efflux systems were inhibited, sulfasalazine demonstrated significantly higher permeability than metoprolol, indicating that, without the involvement of active efflux transport, this drug has the innate potential to exhibit high permeability and to be classified as a Class II compound. Even inhibition of only one out of the two relevant efflux systems revealed this potential, as permeability similar to that of metoprolol was obtained under these conditions (metoprolol is a FDA standard for the low-high permeability class boundary). However, sulfasalazine alone, with no inhibition at all, displayed intestinal permeability significantly lower than that of metoprolol. Because the net permeability of all transport mechanisms is low, sulfasalazine should be classified as a low-permeability compound. Hence, the data clearly show that sulfasalazine susceptibility to efflux transport mediated by MRP2 and BCRP shifts sulfasalazine BCS classification from II to IV.

Sulfasalazine susceptibility to both MRP2- and BCRP-mediated intestinal efflux demonstrated in this paper presents a case of overlapping substrate specificities between different efflux transporters. Recently, Takenaka et al. (24) reported such an overlap between BCRP and the MRP family for purine analog drug. It is necessary to determine the contribution of each transporter to the overall efflux process because such information allows the prediction of changes in membrane permeability when the functions of transporters are altered, e.g., by genetic polymorphism, disease state, or drug-drug interactions. In the case of sulfasalazine, altering the function of efflux transporters may lead to an increase in sulfasalazine absorption from the small intestine, thereby leading to a decrease in the amount of drug that reaches the colon and is activated, potentially resulting in an overall decrease in its therapeutic effect. Sulfasalazine-increased transepithelial permeability observed in the presence of the nonsteroidal anti-inflammatory drug indomethacin may be a relevant example for such a scenario. Additionally, sulfasalazine being a cosubstrate may be significant with regard to segmental-dependent transporter expression levels. We have recently shown that the
low expression of P-gp in the proximal regions of the small intestine leads to a minimal role for this transporter in the absorption of P-gp substrates from these segments, enabling a window for such drug compounds to be relatively well absorbed following oral administration (5). However, when more than one transporter is involved in the efflux process, low expression levels in different segments may be compensated for by the presence of the other relevant transporter. Particularly in the case of sulfasalazine, MRP2 expression was recently shown to gradually decrease from the proximal to the distal small intestinal segments (16), whereas BCRP levels remain constant along the small intestine. Overall, the data presented in this paper demonstrate the mechanism behind sulfasalazine low intestinal absorption, which is responsible for its colonic targeting and therapeutic action.

REFERENCES


