International Journal of Pharmaceutics 386 (2010) 216–220

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

MRP2 mediated drug–drug interaction: Indomethacin increases sulfasalazine absorption in the small intestine, potentially decreasing its colonic targeting

Arik Dahan[a], Gordon L. Amidon[b,*]

[a] Department of Clinical Pharmacology, School of Pharmacy, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
[b] The University of Michigan College of Pharmacy, Department of Pharmaceutical Sciences, 428 Church Street, Ann Arbor, MI 48109, USA

ARTICLE INFO

Article history:
Received 20 August 2009
Received in revised form
17 November 2009
Accepted 18 November 2009
Available online 26 November 2009

Keywords:
Intestinal absorption
Molecular biopharmaceutics
Efflux transport
Drug–drug interaction
Passive/active GI wall permeability

ABSTRACT

We have recently shown that efflux transport, mediated by multidrug resistance-associated protein 2 (MRP2) and breast cancer resistance protein (BCRP), is responsible for sulfasalazine low-permeability in the small intestine, thereby enabling its colonic targeting and therapeutic action. The purpose of the present study was to evaluate the potential pharmacokinetic interaction between indomethacin and sulfasalazine, in the mechanism of efflux transporter competition. The concentration-dependent effects of indomethacin on sulfasalazine intestinal epithelial transport were investigated across Caco-2 cell monolayers, in both apical to basolateral (AP–BL) and BL–AP directions. The interaction was then investigated in the *in situ* single-pass rat jejunal perfusion model. Sulfasalazine displayed 30-fold higher BL–AP than AP–BL Caco-2 permeability, indicative of net mucosal secretion. Indomethacin significantly increased AP–BL and decreased BL–AP sulfasalazine Caco-2 transport, in a concentration-dependent manner, with IC_{50} values of 75 and 196 μM respectively. In the rat model, higher sulfasalazine concentrations resulted in higher intestinal permeability, consistent with saturation of efflux transporter. Without indomethacin, sulfasalazine demonstrated low rat jejunal permeability (vs. metoprolol). Indomethacin significantly increased sulfasalazine P_{eff}, effectively shifting it from BCS (biopharmaceutics classification system) Class IV to II. In conclusion, the data indicate that concomitant intake of indomethacin and sulfasalazine may lead to increased absorption of sulfasalazine in the small intestine, thereby reducing its colonic concentration and potentially altering its therapeutic effect.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

ATP-dependent efflux transporters, such as P-glycoprotein (P-gp; ABCB1), multidrug resistance-associated protein 2 (MRP2; ABCC2) and breast cancer resistance protein (BCRP; ABCG2), have been shown to play significant role in drug absorption, distribution and clearance processes, as well as in drug–drug and drug–food interactions (Dahan and Altman, 2004; Krishnamurthy and Schuetz, 2006; Kwon et al., 2004; Raub, 2006; Takano et al., 2006). In the oral absorption level, these transporters are present on the apical brush border membrane of gut enterocytes, and actively cause efflux of drugs from gut epithelial cells back into the intestinal lumen. While intestinal efflux transport processes present a significant barrier to drug absorption following oral administration and the challenge is to overcome it, we have recently revealed an opposite scenario, in which intestinal efflux is acting in favor of drug delivery. We have shown that intestinal efflux transport mediated by MRP2 and BCRP, but not P-gp, shifts sulfasalazine permeability from high to low, resulting in its very low (2–13%; Klotz, 1985; Sandborn and Hanauer, 2003) intestinal absorption, which essentially enables its colonic targeting and therapeutic action (Dahan and Amidon, 2009c).

The nonsteroidal anti-inflammatory drug indomethacin is a known inhibitor of the MRP family, including MRP2, which has been shown to alter the pharmacokinetics of drugs and to cause drug–drug interactions via MRP inhibition (Dahan et al., 2009b; El-Sheikh et al., 2007; Lindahl et al., 2004; Nozaki et al., 2007). The purpose of this study was to investigate the potential pharmacokinetic interaction between indomethacin and sulfasalazine, in the mechanism of efflux transporter competition. Such interaction 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 activated, potentially resulting in an overall decrease in its therapeutic effect. The concentration-dependent effects of indomethacin on sulfasalazine bidirectional intestinal permeability were investigated across Caco-2 cell monolayers. The interaction was then investigated in the *in situ* rat jejunal perfusion model. Since both drugs may be prescribed in the same medical conditions e.g. rheumatoid arthritis, awareness of this potential interaction is prudent.

* Corresponding author. Tel.: +1 734 764 2226; fax: +1 734 763 6282.
E-mail addresses: gla.cmdt@umich.edu, glamidon@umich.edu (G.L. Amidon).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.11.021

2. Materials and methods

2.1. Materials

Sulfasalazine, indomethacin, metoprolol, phenol red, lucifer yellow, MES buffer, HEPES buffer, glucose, $CaCl_2$, $MgCl_2$ and trifluoroacetic acid were purchased from Sigma Chemical Co. (St. Louis, MO). KCl and NaCl were obtained from Fisher Scientific Inc. (Pittsburgh, PA). Acetonitrile and water (Acros Organics, Geel, Belgium) were HPLC grade. Physiological saline solution was purchased from Hospira Inc. (Lake Forest, IL). All other chemicals were of analytical reagent grade.

2.2. Caco-2 permeability studies

Caco-2 cells (passages 22–27) from American Type Culture Collection (Rockville, MD) were routinely maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen Corp., Carlsbad, CA) containing 10% fetal bovine serum, 1% nonessential amino acids, 1 mM sodium pyruvate, and 1% L-glutamine. Cells were grown in an atmosphere of 5% CO_2 and 90% relative humidity at 37 °C. The DMEM medium was routinely replaced by fresh medium every 3 days. Cells were passaged upon reaching approximately 80% confluence.

Transepithelial transport studies were performed in a method described previously (Dahan and Amidon, 2009a). In brief, cells 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 TEER measurements were performed on all monolayers (Millicell-ERS epithelial Voltohmmeter, Millipore Co., Bedford, MA). Monolayers with apparent mannitol and lucifer yellow permeability $<3 \times 10^{-7}$ cm/s, and TEER values $>350\ \Omega\ cm^2$ were used for all studies. On the experiment day, the monolayers were rinsed and incubated for 20 min with a blank transport buffer (1 mM $CaCl_2$, 0.5 mM $MgCl_2 \cdot 6H_2O$, 145 mM NaCl, 3 mM KCl, 1 mM NaH_2PO_4, 5 mM D-glucose, and 5 mM HEPES). The same pH was used in both apical and basolateral sides (pH 7.4) in order to maintain a 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 and replaced with sulfasalazine uptake buffer solution, with or without indomethacin. Throughout the experiment, the transport plates were kept in a shaking incubator (50 rpm) at 37 °C. Samples were taken from the receiver side at various time points up to 120 min (100 μl), and similar volumes of blank buffer were added following each sample withdrawal. At the last time point samples were taken from the donor side as well, to confirm mass balance. All Caco-2 monolayers were checked for confluence by measuring the TEER before and after the transport study.

2.3. Determination of the IC_{50} of indomethacin on sulfasalazine transport

The concentration-dependent effects of indomethacin (1–1000 μM) on the absorptive (AP–BL) and secretory (BL–AP) directions transport of sulfasalazine (0.1 mM) were investigated. Michaelis–Menten parameters and IC_{50} values were then determined using nonlinear regression according to the Hill equation with GraphPad Prism 4.01 (GraphPad Software Inc., San Diego, CA), as previously described (Dahan et al., 2009a).

2.4. Single-pass intestinal perfusion (SPIP) studies in rats

All animal experiments were conducted using protocols approved by the University of Michigan Committee of Use and Care of Animals (UCUCA) and the Ben-Gurion University of the Negev Animal Use and Care Committee. Male Wistar rats (Charles River, IN) weighing 250–300 g were used for all perfusion studies. Prior to each experiment, rats were fasted overnight 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 (Dahan and Amidon, 2009b; Kim et al., 2006). In brief, rats were anesthetized with an i.m. injection of 1 ml/kg ketamine–xylazine solution (9%:1%, respectively) and placed on a heated surface maintained at 37 °C. The abdomen was opened by a midline incision, and a jejunal segment (10 cm) was carefully exposed and cannulated at both ends with flexible PVC tubing (2.29 mm i.d., inlet tube 40 cm, outlet tube 20 cm, Fisher Scientific Inc.). 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, and was kept moist with 37 °C physiological solution.

At the start of the study, perfusion solution containing sulfasalazine (25 or 100 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 indomethacin (0.28 mM), was perfused through the intestinal segment (Watson-Marlow Pumps 323S, Watson-Marlow Bredel Inc., Wilmington, MA), at a flow rate of 0.2 ml/min. Metoprolol was co-perfused 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 (Dahan et al., 2009c; Kim et al., 2006). The perfusion buffer was first perfused for 1 h to assure steady-state, and then, samples were taken in 10 min intervals for 1 h. Following the termination of the experiment, the length of each perfused intestinal segment was accurately measured.

2.5. 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 drug concentration in the donor side, and A is the monolayer growth surface area (1.12 cm^2).

The efflux ratio (ER) was determined by calculating the ratio of P_{app} in the secretory (BL–AP) direction divided by the absorptive (AP–BL) direction P_{app}:

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

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

$$P_{eff} = \frac{-Q\ \ln(C'_{out}/C'_{in})}{2\pi RL}$$

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 rat perfusion studies was determined using phenol red (0.1 mg/ml), a nonabsorbed, nonmetabolized marker that was co-perfused with the other drugs (Dahan et al., 2009c; Kim et al., 2006). The measured C_{out}/C_{in} ratio was corrected for water flux 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 $C_{in\ phenol\ red}$ and $C_{out\ phenol\ red}$ are equal to the concentration of phenol red in the inlet and the outlet samples, respectively.

2.6. 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 an HPLC system (Waters 2695 Separation Module) with a PDA UV detector (Waters 2996). Samples were filtered (Unifilter® 96 wells microplate 0.45 μm filters, Whatman Inc., Florham Park, NJ), and Caco-2 medium aliquots of 70 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 mm × 100 mm column (Waters Co., Milford, MA); a gradient mobile phase, going from 75:25% to 40:60%, v/v 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 rate of 1 ml/min at 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 ($R^2 > 0.99$). For all compounds, the minimum quantifiable concentrations were ~50 ng/ml, and the inter- and intra-day coefficients of variation were <1.0% and 0.5%, respectively.

2.7. Statistical analysis

All Caco-2 experiments were performed in triplicates (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 non-parametric Kruskal–Wallis test was used for multiple comparisons, and the two-tailed non-parametric Mann–Whitney *U*-test for two-group comparison when appropriate. A p value of less than 0.05 was termed significant.

3. Results and discussion

While for the vast majority of drugs systemic absorption following oral administration is desired, the case of sulfasalazine presents a unique scenario; low absorption due to extensive efflux essentially enables sulfasalazine colonic targeting and activation by colonic bacterial azo reductases, thereby enabling its therapeutic action. Hence, decreased efflux transport e.g. by concomitant intake of indomethacin, may lead to altered colonic concentrations and potentially therapeutic action of sulfasalazine. Since both drugs may be prescribed in the same medical conditions e.g. rheumatoid arthritis, we have investigated this potential drug–drug interaction.

3.1. The effect of indomethacin on sulfasalazine Caco-2 permeability

The flux of sulfasalazine across Caco-2 cell monolayers in the AP–BL and BL–AP directions, in the presence vs. absence of 500 μM indomethacin, is presented in Fig. 1. In the absence of indomethacin, sulfasalazine exhibited 30-fold higher BL–AP than AP–BL Caco-2 permeability, indicative of extensive net mucosal secretion. It can be seen that indomethacin caused significant increase in AP–BL and decrease in BL–AP sulfasalazine Caco-2 transport, reducing its efflux ratio (ER) from 30 to 4. The Caco-2 expression of MRP2 was validated using Western blot analysis (Dahan et al., 2009a,b), confirming that this efflux transporter was 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 absorptive (AP–BL) and secretory (BL–AP) directions by indomethacin are shown in Fig. 2. Indomethacin displayed a concentration-dependent inhibition of sulfasalazine mucosal secretion in both directions; the IC_{50} values obtained for the AP–BL and BL–AP inhibition were 75.1 μM (31.0–182.0) and 196.0 μM (92.3–416.3), respectively. These IC_{50} values indicate a relevant concentration region; indomethacin lowest single-unit oral dose, 25 mg, together with the acceptable initial gastric volume following drug intake, 250 ml, yield a GI concentration of 100 μg/ml or 280 μM, which is significantly higher than the AP–BL IC_{50} and in the region of the BL–AP IC_{50} value.

Sulfasalazine Caco-2 efflux ratio (ER) values obtained in the presence of different indomethacin concentrations are presented in Fig. 3. Overall, indomethacin was able to greatly decrease sulfasalazine ER, from 30 in the absence of indomethacin to 4 in the lower plateau region (starting at 500 μM indomethacin). How-

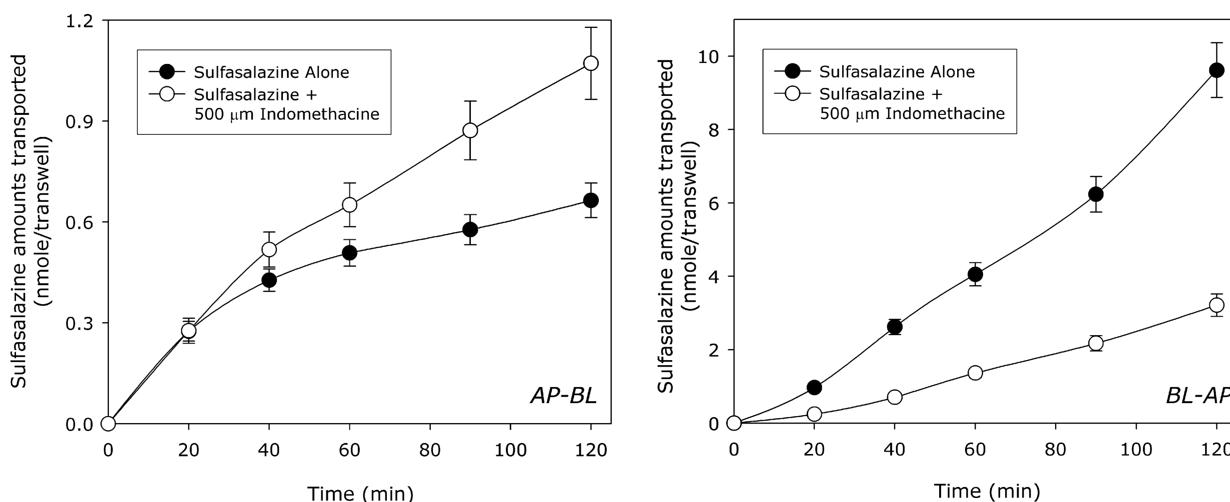

Fig. 1. Sulfasalazine (0.1 mM) flux across Caco-2 cell monolayers in the absorptive (AP–BL; *left*) and secretory (BL–AP; *right*) directions, in the absence vs. presence of 500 μM indomethacin. Data are presented as means ± SD; $n = 3$ in each experimental group.

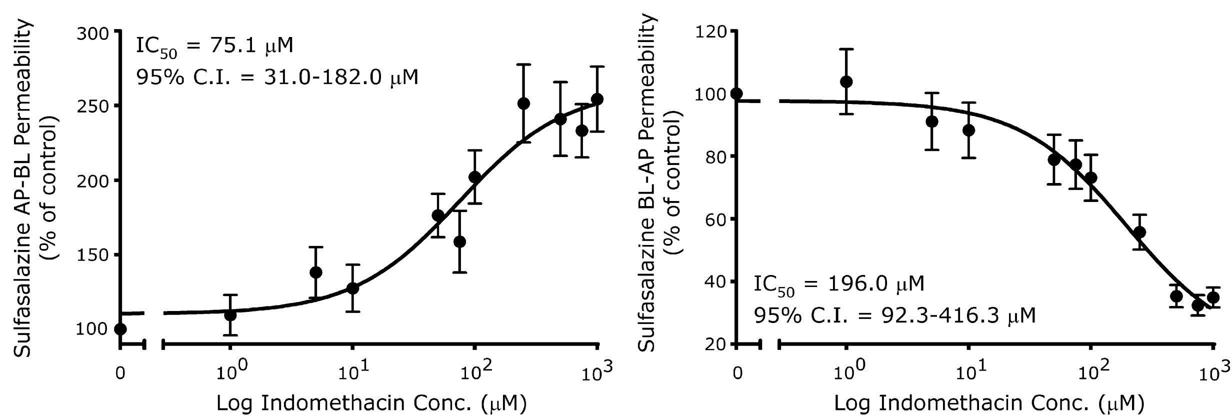

Fig. 2. The dose–response curves for the inhibition of sulfasalazine (0.1 mM) mucosal secretion across Caco-2 cell monolayers in the absorptive (AP–BL; *left*) and secretory (BL–AP; *right*) directions by indomethacin (1–1000 μM). Data are presented as means ± SD; n = 3 in each experimental group.

ever, it can be seen that ER values lower than 4 could not be achieved, pointing out the continuation of an efflux process even in the presence of high indomethacin concentrations. Sulfasalazine transepithelial efflux transport has been shown to be mediated not only by MRP2 but by BCRP as well (Dahan and Amidon, 2009c; Liang et al., 2000; Mols et al., 2005; Zaher et al., 2006). Hence, even when the MRP2 mediated efflux was inhibited by indomethacin, an efflux process mediated by BCRP was still active, resulting in the sulfasalazine ER = 4 in the lower plateau region. Indeed, the ER value of 4 is identical to that obtained in the presence of the MRP2 inhibitor MK-571 (Dahan and Amidon, 2009c), i.e. when only BCRP mediated efflux was active. When both MRP2 and BCRP inhibitors were simultaneously present, an ER of 1 was obtained, indicating the complete abolishment of sulfasalazine efflux transport.

3.2. The effect of indomethacin on sulfasalazine rat jejunal permeability

Sulfasalazine permeability coefficients (P_{eff}) obtained following *in situ* perfusion in the rat jejunum, at different initial drug concentrations, and in the presence vs. absence of indomethacin, are presented in Fig. 4. Consistent with saturation of efflux transport, sulfasalazine exhibited non-linear intestinal absorption, showing increased permeability with increasing starting drug concentration. In the absence of indomethacin, at both starting drug concentrations investigated, sulfasalazine displayed low-permeability in the rat jejunum as compared to metoprolol, an FDA standard for the low–high permeability class boundary. The presence of 280 μM indomethacin significantly increased sulfasalazine permeability, bringing it to the level of metoprolol, i.e. the low–high permeability class boundary. As denoted above (Section 3.1), the value for indomethacin concentration, 280 μM, was calculated based on the drug's lowest single-unit oral dose (25 mg) and the acceptable gastric volume following oral drug ingestion (250 ml), thus representing a relevant GI concentration. Hence, it was confirmed that this drug–drug interaction occurs not only *in vitro* in a cell culture model but in the rat as well.

With respect to the biopharmaceutics classification system (BCS) (Amidon et al., 1995; Martinez and Amidon, 2002), the data presented in this paper suggest that concomitant intake of sulfasalazine with indomethacin may shift sulfasalazine classification from IV to II. This result is in corroboration with our previous finding that without the involvement of active efflux transport, sulfasalazine has the innate potential to exhibit high permeability; however, sulfasalazine susceptibility to extensive efflux transport shifts its permeability from high to low (Dahan and Amidon, 2009c). Since the net permeability of all transport mechanisms is low, sulfasalazine should be classified as a low-permeability compound. Yet, when the functions of the relevant efflux transporters are

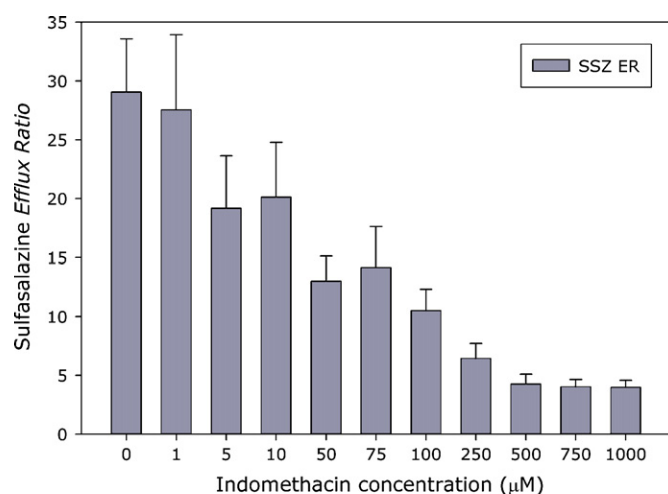

Fig. 3. The efflux ratios (ER; $P_{aap\,BL–AP}/P_{aap\,AP–BL}$) obtained for sulfasalazine transport across Caco-2 cell monolayers in the presence of different indomethacin concentrations (1–1000 μM). Data are presented as means ± SD; n = 3 in each experimental group.

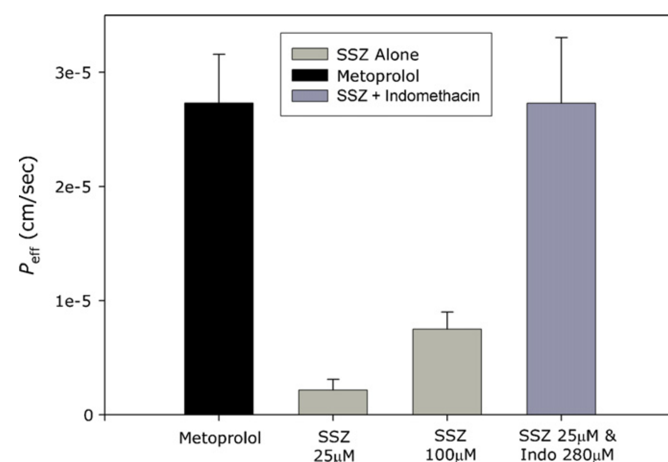

Fig. 4. The permeability coefficients (P_{eff}; cm/s) obtained for sulfasalazine (SSZ) compared with metoprolol (simultaneously perfused) following *in situ* single-pass rat jejunal perfusion, in different starting sulfasalazine concentrations (25 and 100 μM), and in the absence vs. presence of indomethacin (280 μM). Data are presented as means ± SD; sulfasalazine, n = 4 in each experimental group; metoprolol, n = 12.

altered, e.g. by genetic polymorphism (Yamasaki et al., 2008), disease state or drug–drug interactions, the situation may indeed be less predictable.

4. Conclusions

In conclusion, the data presented in this paper show that concomitant intake of indomethacin and sulfasalazine may lead to increased permeability of sulfasalazine in the small intestine *via* MRP2 inhibition, potentially decreasing the colonic concentrations of the drug, thereby altering its therapeutic action. Since both drugs may be prescribed in the same medical conditions e.g. rheumatoid arthritis, especially in the elderly subpopulation, awareness of this potential interaction is prudent.

References

Amidon, G.L., Lennernas, H., Shah, V.P., Crison, J.R., 1995. A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res. 12, 413.

Dahan, A., Altman, H., 2004. Food–drug interaction: grapefruit juice augments drug bioavailability – mechanism, extent and relevance. Eur. J. Clin. Nutr. 58, 1.

Dahan, A., Amidon, G.L., 2009a. Grapefruit juice and its constituents augment colchicine intestinal absorption: potential hazardous interaction and the role of P-glycoprotein. Pharm. Res. 26, 883–892.

Dahan, A., Amidon, G.L., 2009b. Segmental dependent transport of low permeability compounds along the small intestine due to P-glycoprotein: the role of efflux transport in the oral absorption of BCS class III drugs. Mol. Pharm. 6, 19–28.

Dahan, A., Amidon, G.L., 2009c. 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.

Dahan, A., Sabit, H., Amidon, G.L., 2009a. The H2 receptor antagonist nizatidine is a P-glycoprotein substrate: characterization of its intestinal epithelial cell efflux transport. AAPS J. 11, 205–213.

Dahan, A., Sabit, H., Amidon, G.L., 2009b. Multiple efflux pumps are involved in the transepithelial transport of colchicine: combined effect of P-gp and MRP2 leads to decreased intestinal absorption throughout the entire small intestine. Drug Metab. Dispos. 37, 2028–2036.

Dahan, A., West, B.T., Amidon, G.L., 2009c. Segmental-dependent membrane permeability along the intestine following oral drug administration: evaluation of a triple single-pass intestinal perfusion (TSPIP) approach in the rat. Eur. J. Pharm. Sci. 36, 320–329.

El-Sheikh, A.A.K., van den Heuvel, J.J.M.W., Koenderink, J.B., Russel, F.G.M., 2007. Interaction of nonsteroidal anti-inflammatory drugs with multidrug resistance protein (MRP) 2/ABCC2- and MRP4/ABCC4-mediated methotrexate transport. J. Pharmacol. Exp. Ther. 320, 229–235.

Kim, J.S., Mitchell, S., Kijek, P., Tsume, Y., Hilfinger, J., Amidon, G.L., 2006. The suitability of an in situ perfusion model for permeability determinations: utility for BCS class I biowaiver requests. Mol. Pharm. 3, 686–694.

Klotz, U., 1985. Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-aminosalicylic acid. Clin. Pharmacokinet. 10, 285–302.

Krishnamurthy, P., Schuetz, J.D., 2006. Role of ABCG2/BCRP in biology and medicine. Annu. Rev. Pharmacol. Toxicol. 46, 381–410.

Kwon, H., Lionberger, R.A., Yu, L.X., 2004. Impact of P-glycoprotein-mediated intestinal efflux kinetics on oral bioavailability of P-glycoprotein substrates. Mol. Pharm. 1, 455–465.

Liang, E., Proudfoot, J., Yazdanian, M., 2000. Mechanisms of transport and structure–permeability relationship of sulfasalazine and its analogs in Caco-2 cell monolayers. Pharm. Res. 17, 1168–1174.

Lindahl, A., Sjoberg, A., Bredberg, U., Toreson, H., Ungell, A.-L., Lennernas, H., 2004. Regional intestinal absorption and biliary excretion of fluvastatin in the rat: possible involvement of MRP2. Mol. Pharm. 1, 347–356.

Martinez, M.N., Amidon, G.L., 2002. A mechanistic approach to understanding the factors affecting drug absorption: a review of fundamentals. J. Clin. Pharmacol. 42, 620–643.

Mols, R., Deferme, S., Augustijns, P., 2005. Sulfasalazine transport in in-vitro, ex-vivo and in-vivo absorption models: contribution of efflux carriers and their modulation by co-administration of synthetic nature-identical fruit extracts. J. Pharm. Pharmacol. 57, 1565–1573.

Nozaki, Y., Kusuhara, H., Kondo, T., Iwaki, M., Shiroyanagi, Y., Nakayama, H., Horita, S., Nakazawa, H., Okano, T., Sugiyama, Y., 2007. Species difference in the inhibitory effect of nonsteroidal anti-inflammatory drugs on the uptake of methotrexate by human kidney slices. J. Pharmacol. Exp. Ther. 322, 1162–1170.

Raub, T.J., 2006. P-glycoprotein recognition of substrates and circumvention through rational drug design. Mol. Pharm. 3, 3–25.

Sandborn, W.J., Hanauer, S.B., 2003. The pharmacokinetic profiles of oral mesalazine formulations and mesalazine pro-drugs used in the management of ulcerative colitis. Aliment. Pharmacol. Ther. 17, 29–42.

Takano, M., Yumoto, R., Murakami, T., 2006. Expression and function of efflux drug transporters in the intestine. Pharmacol. Ther. 109, 137.

Yamasaki, Y., Ieiri, I., Kusuhara, H., Sasaki, T., Kimura, M., Tabuchi, H., Ando, Y., Irie, S., Ware, J.A., Nakai, Y., Higuchi, S., Sugiyama, Y., 2008. Pharmacogenetic characterization of sulfasalazine disposition based on NAT2 and ABCG2 (BCRP) gene polymorphisms in humans. Clin. Pharmacol. Ther. 84, 95–103.

Zaher, H., Khan, A.A., Palandra, J., Brayman, T.G., Yu, L., Ware, J.A., 2006. Breast cancer resistance protein (Bcrp/abcg2) is a major determinant of sulfasalazine absorption and elimination in the mouse. Mol. Pharm. 3, 55–61.