Role of organic anion transporting polypeptides (OATPs) for cellular mesalazine (5-aminosalicylic acid) uptake

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Running Title Page

a. Running Title:

OATPs and cellular mesalazine uptake

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d. Non-standard abbreviations: HEK cells, human embryonic kidney cells; OATPs, organic anion transporting polypeptides; 5-ASA, 5-aminosalicylic acid; SLCO, solute carrier family of the OATPs; ATR, ATM-and-Rad3-related kinase; BSP, sulfobromophthalein; $V_{max}$, maximum transport rate; $K_m$, Michaelis-Menten constant; ANOVA, analysis of variance
Abstract

Therapeutic effects and metabolism of mesalazine (5-aminosalicylic acid; 5-ASA) in patients with inflammatory bowel disease requires intracellular accumulation of the drug in intestinal epithelial cells and hepatocytes. The molecular mechanisms of mesalazine uptake into cells have not been characterized so far. Using HEK cells stably expressing uptake transporters of the organic anion transporting polypeptide family (OATPs), which are expressed in human intestine and/or liver, we found that mesalazine uptake is mediated by OATP1B1, OATP1B3 and OATP2B1, but not by OATP1A2 and OATP4A1. Moreover, genetic variations (*1b, *5, *15) in the SLCO1B1 gene encoding OATP1B1 reduced the K_m value for mesalazine uptake from 55.1 to 16.3, 24.3 and 32.4 µM, respectively, and the respective V_max values. Finally, budesonide, cyclosporin A and rifampin were identified as inhibitors of OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine uptake. These in vitro data indicate that OATP-mediated uptake and its modification by genetic factors and comedications may play a role for mesalazine effects.
Introduction

Mesalazine (5-aminosalicylic acid, 5-ASA, mesalamine) represents a drug of first choice for the treatment of mild to moderate ulcerative colitis. Moreover, long-term use of mesalazine in patients with chronic inflammatory bowel disease reduces the risk of development of colorectal cancer in patients with ulcerative colitis (Velayos, et al., 2005). Recent in vitro data indicate that an important underlying mechanism of the chemopreventive action of mesalazine is that mesalazine causes cells to reversibly accumulate in the S-phase and activate an ATR-dependent checkpoint with subsequent increase in the maintenance of genomic stability and counteracting carcinogenesis (Luciani, et al., 2007; Koelink, et al., 2010).

Mesalazine is believed to act intracellularly in epithelial cells of the gut with topical delivery from the luminal side of the intestine (Schwab and Klotz, 2001). Sufficient delivery of the active drug to the inflamed area is an important determinant of therapeutic efficacy, whereas the extent of drug absorption into the systemic circulation might influence tolerability (Schwab and Klotz, 2001; Klotz and Schwab, 2005). In patients with ulcerative colitis there was indeed a negative correlation between mucosal concentrations of mesalazine and the severity of colonic inflammation (Frieri, et al., 2000). Absorbed mesalazine is metabolized in the intestinal epithelium and in liver to pharmacologically inactive N-acetyl-mesalazine (Schwab and Klotz, 2001).

Uptake transporters such as members of the organic anion transporting polypeptide family (OATPs, gene symbol: SLCO), which are located in the luminal membrane of enterocytes and in the basolateral membrane of hepatocytes, are well-recognized determinants of drug absorption, distribution and effects (Nies, et al., 2009; Fahrmayr, et al., 2010). OATPs transport a broad spectrum of endogenous compounds and drugs (Fahrmayr, et al., 2010). OATP1B1, OATP1B3 and OATP2B1 are the three major hepatic OATPs mediating uptake of their substrates from the portal venous blood into the hepatocytes. Genetically
determined variations in the SLCO1B1 gene affecting OATP1B1 function (e.g. SLCO1B1*1b, *5, *15) have a major impact on plasma concentrations, effects and side effects of multiple drugs including HMG-CoA reductase inhibitors (Marzolini, et al., 2004; Link, et al., 2008; Fahrmayr, et al., 2010; Niemi, 2010). Moreover, inhibition of specific uptake transporters by concomitantly administered drugs is an important mechanism of drug-drug interactions [for review see e.g. (Kindla, et al., 2009; Fahrmayr, et al., 2010)].

OATP1A2 and OATP2B1 expression has also been reported for enterocytes in the small intestine thereby affecting drug absorption (Kobayashi, et al., 2003; Glaeser, et al., 2007). For example, coadministration of grapefruit juice with the antihistaminic drug fexofenadine leads to significantly reduced plasma concentrations of the OATP substrate fexofenadine (Glaeser, et al., 2007). At the cellular level, inhibition of OATP1A2-mediated fexofenadine uptake by naringin, a major constituent of grapefruit juice, was identified as the underlying mechanism (Bailey, et al., 2007). OATP1A2, OATP1B1, OATP1B3 and OATP2B1 expression has also been reported for human colon (Meier, et al., 2007; Hilgendorf, et al., 2007) and OATP1B3 expression for colorectal cancer (Ballestero, et al., 2006; Lee, et al., 2008). Interestingly, Wojtal et al. recently reported an upregulation of OATP2B1 and OATP4A1 in inflamed compared to noninflamed tissue samples from terminal ileum and colon from patients with Crohn’s disease and a significant upregulation of OATP2B1 and OATP4A1 in paired colonic specimen from patients with ulcerative colitis (Wojtal, et al., 2009).

Currently there are no published data on molecular mechanisms of mesalazine uptake into cells. This study aimed at answering the following questions: (1) Is mesalazine uptake into cells mediated by members of the OATP family? (2) Do genetic variations in the SLCO1B1 gene affect mesalazine uptake? (3) Could concomitantly administered drugs affect intracellular mesalazine concentrations via inhibition of OATP transporters? These questions were addressed by HEK cell lines stably expressing human OATP1A2,
OATP1B1, OATP1B3, OATP2B1 and OATP4A1 as well as by HEK cells expressing the polymorphic OATP1B1 variants OATP1B1*1b (SLCO1B1c.388A>G, OATP1B1p.N130D), *5 (SLCO1B1c.521T>C, OATP1B1p.V174A) and *15 (SLCO1B1c.388A>G+521T>C, OATP1B1p.N130D+V174A).
Materials and Methods

Chemicals

[\(^{3}\)H]Sulfobromophthalein ([\(^{3}\)H]BSP; 7585 GBq/mmol) was obtained from Hartmann Analytic (Braunschweig, Germany). Unlabelled sulfobromophthalein and poly-D-lysine hydrobromide were purchased from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). [\(^{3}\)H]Mesalazine (26.3 Ci/mmol) was purchased from ViTrax (Placentia, CA), unlabelled mesalazine was obtained from Sigma-Aldrich (Taufkirchen, Germany). G418 (Geniticin) disulfate and Hygromycin were from Invitrogen (Groningen, Netherlands).

Cloning of the \textit{SLC01A2} cDNA encoding human OATP1A2

The \textit{SLCO1A2} coding sequence (NM_134431.3) was cloned by reverse transcription reaction and subsequent polymerase chain reaction from human brain cDNA (Human Total RNA Master Panel II, Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France) into the pcDNA3.1(+) vector (Invitrogen GmbH, Karlsruhe, Germany) and subsequently subcloned into the retroviral vector pQCXIN (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France). HEK293 cells were transfected with the plasmid pQCXIN-OATP1A2 using a retroviral gene transfer and expression kit (Takara Bio Europe/Clontech, Saint-Germain-en-Laye, France). After treatment with geneticin (500 µg/ml), single colonies were selected and characterized for \textit{SLCO1A2} mRNA expression using real-time PCR as previously described (Mandery, et al., 2010). The primer for real-time PCR analysis of \textit{SLCO1A2} mRNA were forward: 5’-AAGACCAACGCAGGATCCAT-3’ and reverse: 5’-GAGTTTCACCCATTCCACGTACA-3’ with a resulting amplicon size of 101 base pairs. The primers for the housekeeping gene \textit{\(\beta\)-actin} were forward: 5’-TGACGGGGTCACCCACACTGTGCCCATCTA-3’ and reverse: 5’-CTAGAAGCATTGCGGTGGACGATGGAGGG-3’ with a resulting amplicon size of 661 base pairs.
Cloning of the \textit{SLC04A1} cDNA encoding human OATP4A1

The \textit{SLCO4A1} cDNA encoding human OATP4A1 was cloned by a RT-PCR-based approach using kidney total RNA (Multiple cDNA panel from Clontech, Heidelberg, Germany) as template for the single-strand cDNA synthesis. Single-strand cDNA synthesis was performed as described (König, et al., 1999). For the amplification of the full-length cDNA the primer pair oOATP4A1-5´.for (5´-TGA AGC CTC GAG GTC ACC AG-3´) located in front of the ATG start codon and the reverse primer oOATP4A1-RT.rev (5´-GCG GTG GTC AGA CGC TGC T-3´) located behind the stop codon were used. The amplified cDNA fragment was subcloned into the vector pCR2.1.TOPO (Invitrogen) and the sequence was verified. Two base-pair exchanges resulting in amino acid exchanges were corrected using the multisite-directed mutagenesis kit (Stratagene, Amsterdam, The Netherlands) according manufacturer’s instructions. The resulting \textit{SLCO4A1} cDNA encodes for an OATP4A1 protein identical to the one encoded by the reference sequence (NM_016354.3). This cDNA was subcloned into the vector pcDNA3.1(+) (Invitrogen) and used for transfection.

Generation of stably transfected cells

Generation and validation of HEK-OATP1B1, HEK-OATP1B3 and HEK-OATP2B1 cells as well as the cells expressing the OATP1B1 variant *5 have been described before (Bachmakov, et al., 2008). HEK293 cells stably expressing the OATP1B1 variants OATP1B1*1b and OATP1B1*15 as well as HEK293 cells expressing human OATP1A2 and OATP4A1 were established as described (Seithel, et al., 2007). Cell clones were screened by quantitative RT-PCR for mRNA expression and the protein synthesis was verified by immunoblot analysis (Seithel, et al., 2007). Finally, the cell clones with the highest mRNA and corresponding protein expression were used for subsequent transport experiments.
Uptake and uptake inhibition studies

Uptake assays have been performed as previously described (Seithel, et al., 2007). In brief, 700,000 HEK-OATP cells (stably expressing an OATP protein) or the respective HEK-Co cells (HEK-Co/418 or HEK-Co/Hy; control cell lines transfected with the empty expression vectors pcDNA3.1(+) and pcDNA3.1/Hygro, respectively) were seeded in 12-well plates [coated with 0.1 mg/ml poly-D-lysine (Greiner Bio-One, Frickenhausen, Germany)] and cultured for two days. 24 h before uptake or uptake inhibition experiments, cells were induced with 10 mM sodium butyrate (Merck KGaA, Darmstadt, Germany) in order to obtain higher levels of recombinant protein expression (Cui, et al., 1999). Before uptake experiments, cells were washed with prewarmed uptake buffer (142 mM NaCl, 5 mM KCl, 1 mM K₂HPO₄, 1.2 mM MgSO₄, 1.5 mM CaCl₂, 5 mM glucose and 12.5 mM HEPES, pH 7.3). [³H]BSP or [³H]mesalazine was dissolved in uptake buffer and unlabelled BSP or mesalazine was added to reach the mentioned final concentrations. For uptake inhibition experiments mesalazine (as inhibitor of OATP-mediated BSP uptake) or several drugs (as inhibitors of OATP-mediated mesalazine uptake) were added in the respective concentrations into the uptake solution. The cells were incubated with the uptake solution for 10 min and subsequently washed three-times with ice-cold uptake buffer to remove radioactivity bound to the cell membrane. An incubation time of 10 min was used for mesalazine, since experiments have demonstrated linearity of uptake up to 10 min using mesalazine in a concentration of 20 µM. After the cells were lysed with 0.2% SDS, the intracellular accumulation of radioactivity was determined by liquid scintillation counting (Tricarb 2800, Perkin Elmer Life Science GmbH) and the appropriate protein concentrations was determined by a bicinchonic acid assay (BCA protein assay Kit, Thermo Scientific, Bonn, Germany). Kᵢ values for inhibition of OATP1B1-mediated mesalazine uptake were determined using different mesalazine (7.5 µM or 10 µM, 50 µM
and 100 µM) and inhibitor concentrations. For pravastatin and budesonide as inhibitor of OATP1B1-mediated mesalazine uptake 1 µM, 10 µM and 25 µM of the respective drug and for rifampicin and cyclosporin A 0.1 µM, 1 µM, 5 µM and 10 µM were used. These data were analyzed by the method of Dixon, in which the reciprocal velocity (1/V) is plotted against the inhibitor concentration.

**Statistical Analysis**

The uptake experiments were repeated at least two times at different days with n = 3, i.e. for each concentration and time point six (and for control experiments up to twelve) separate wells were investigated. All data are presented as mean ± standard deviation. Transport kinetics were calculated by Prism 3.01 (GraphPad Software, La Jolla USA). The calculated parameters were maximum transport rate (V\(_{\text{max}}\)) and Michaelis-Menten constant (K\(_{\text{m}}\)). K\(_{\text{m}}\) values were calculated using the non-linear regression (One-site binding) curve fit from the same software package. Multiple comparisons were analyzed by ANOVA with subsequent Dunnetts multiple comparison test by using Prism 3.01. A value of P < 0.05 was required for statistical significance.
Results

Inhibition of OATP1A2-, OATP1B1-, OATP1B3- and OATP2B1-mediated BSP uptake by mesalazine

The influence of increasing concentrations of mesalazine on OATP1A2-, OATP1B1-, OATP1B3- and OATP2B1-mediated BSP uptake are shown in Fig. 1. Mesalazine had no significant effect on OATP1A2- and OATP2B1-mediated BSP uptake. A significant, concentration-dependent inhibition of BSP uptake by mesalazine was found for OATP1B1- and OATP1B3-mediated BSP uptake (Fig. 1).

Uptake of mesalazine by OATP1B1, OATP1B3 and OATP2B1

Using 20 μM mesalazine OATP1B1, OATP1B3 and OATP2B1 expressing cells showed an 2.7-, 2.9- and 2.0-fold, respectively, higher intracellular mesalazine accumulation compared to vector control cells (Fig. 2). Fig. 3 shows the kinetics of OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine net uptake. Km values were lowest for OATP1B1-mediated mesalazine uptake (55.1 μM) compared with OATP1B3- and OATP2B1-mediated mesalazine uptake (77.4 μM and 188.9 μM, respectively). No significant differences in mesalazine uptake were found for OATP1A2 and OATP4A1 expressing cells compared to the vector-transfected cells (data not shown).

Impact of genetic variants in the SLCO1B1 gene on OATP1B1-mediated mesalazine uptake

We next determined the impact of genetic variations in the SLCO1B1 gene on OATP1B1-mediated mesalazine uptake. As shown in Fig. 4 and Table 1, we observed lower Km and lower Vmax values (p < 0.01) for mesalazine uptake mediated by the polymorphic variants SLCO1B1*1b, *5 and *15 compared to wild type SLCO1B1*1a (Table 1).
Influence of drugs on OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine uptake

Finally, the impact of known OATP inhibitors or of drugs used for treatment of inflammatory bowel disease on OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine uptake was investigated. Pravastatin (100 μM) significantly inhibited mesalazine uptake mediated by all hepatic OATPs (p < 0.01, Fig. 5). At the lower concentration of 10 μM pravastatin inhibited significantly OATP1B1-mediated mesalazine uptake (p < 0.05, Fig. 5). Azathioprine had only a modest effect on OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine uptake (Fig. 5). Cyclosporin A and rifampin were particularly strong inhibitors of OATP1B1- and OATP1B3-mediated mesalazine uptake, but inhibited also significantly OATP2B1-mediated mesalazine uptake. Interestingly, budesonide at 10 μM and 100 μM inhibited OATP1B1- (p <0.05), OATP1B3- (p < 0.01) and OATP2B1-mediated mesalazine uptake (p < 0.01, Fig. 5). We also analyzed the effect of these drugs (in a concentration of 100 μM) on OATP1B1-, OATP1B3- and OATP2B1-mediated BSP uptake and could confirm these results (data not shown). As shown for OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine uptake, pravastatin, budesonide, cyclosporin A and rifampin significantly inhibited BSP uptake, whereas clarithromycin inhibited only OATP1B1- and OATP1B3-mediated BSP uptake and azathioprine had no significant effect on OATP-mediated BSP uptake. Additional Dixon plot analyses were indicative of competitive inhibition of OATP1B1-mediated mesalazine uptake by pravastatin, budesonide, cyclosporin A and rifampin with Kᵢ values of 7.2, 2.1, 0.7 and 1.0 μM, respectively (data not shown).
Discussion

This is the first study showing that intracellular accumulation of mesalazine is mediated by members of the OATP uptake transporter family, which are expressed in small and large intestine and in the liver. Desired therapeutic effects of mesalazine in the intestine requires a sufficient intracellular accumulation of the drug (Schwab and Klotz, 2001; Luciani, et al., 2007; Koelink, et al., 2010). Based on our data it can be concluded that mesalazine is a substrate for the OATP family members OATP1B1, OATP1B3 and OATP2B1. These transporters are not only the most important OATP uptake transporters in the basolateral membrane of hepatocytes, thus being an essential step for the hepatic elimination of drugs (Kindla, et al., 2009), but they are also expressed in human healthy colon and colorectal cancer (Ballestero, et al., 2006; Meier, et al., 2007; Hilgendorf, et al., 2007; Lee, et al., 2008). Interestingly, Wojtal et al. recently reported an upregulation of OATP2B1 and OATP4A1 in inflamed compared to non-inflamed tissue originating from terminal ileum and colon from patients with Crohn’s disease and a significant upregulation of OATP2B1 and OATP4A1 in paired colonic specimen from patients with ulcerative colitis (Wojtal, et al., 2009), indicating that the upregulation of OATP2B1 could lead to an enhanced accumulation of the OATP2B1 substrate mesalazine in the inflamed tissues.

Previous clinical studies indicate that a fraction of mesalazine and its metabolite N-acetyl-mesalazine are secreted back from the enterocytes into the gut lumen (Goebell, et al., 1993; Layer, et al., 1995). For other drugs, efflux transporters expressed in the luminal membrane of enterocytes such as P-glycoprotein, MRP2 and BCRP are well recognized determinants of intracellular accumulation in the enterocytes and efflux into the gut lumen. The molecular mechanism of secretion of mesalazine back into the gut lumen is still unclear, since recent in vitro data by Xin et al. showed that mesalazine is not a substrate of the efflux pumps P-glycoprotein and MRP2 (Xin, et al., 2006).
Mesalazine shows desired therapeutic effects in inflammatory bowel disease via topical mechanisms in the intestine. Nevertheless, after oral administration or administration in the form of enemas or suppositories mesalazine and its metabolite reach significant concentrations in the systemic circulation [approximately 20 µM for mesalazine; e.g. (Lück, et al., 2009)], which have been linked to the tolerability (e.g. hepatic, renal function) of the drug (Schwab and Klotz, 2001). Intramucosal concentrations of mesalazine can be as high as 2 mM (De Vos, et al., 1992) and portal venous concentrations are expected to be higher than systemic plasma mesalazine concentrations. Our data indicate that mesalazine delivered from the portal venous blood to the liver, will be taken up by OATP1B1, OATP1B3 and OATP2B1 localized in the basolateral membrane of hepatocytes (K_m values 55.1, 77.4 and 188.9 µM, respectively). Genetic variations in the \textit{SLCO1B1} gene encoding for mutated OATP1B1 proteins, in particular the variants \textit{SLCO1B1}*5 and *15, affect plasma concentrations, therapeutic effects and side effects of a broad variety of drugs, including statins and certain oral antidiabetic drugs (Fahrmayr, et al., 2010). For example, recent studies reported a strong dependence of simvastatin acid plasma concentrations on \textit{SLCO1B1} genotype (Pasanen, et al., 2006) and a clear relationship between \textit{SLCO1B1} variants and the risk for statin-induced myopathy (Link, et al., 2008). Our \textit{in vitro} data clearly show an impaired mesalazine uptake by cells stably expressing the variants OATP1B1*1b, *5 and *15 compared to wild type OATP1B1. The clinical consequences of our observation need to be clarified in further studies, but we postulate that mesalazine plasma concentrations and possibly some of its side effects will depend on \textit{SLCO1B1} genotype. To the best of our knowledge, the impact of \textit{SLCO1B1} genotypes on mRNA or protein expression in the colon has not been investigated.

In addition to genetic factors, local drug concentrations at the site of action, drug disposition in the systemic circulation and drug effects can considerably be altered by concomitantly administered drugs. An important mechanism of drug-drug interactions is
inhibition of drug uptake by a second, concomitantly administered drug [for review see e.g. (Fahrmayr, et al., 2010)]. Since we were able to show that mesalazine is a substrate of OATP1B1, OATP1B3 and OATP2B1, we determined the impact of drugs known to inhibit OATPs and of other drugs used for treatment of inflammatory bowel disease on OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine uptake. It should be noted that transporters located in the luminal membrane of enterocytes and in the basolateral membrane of hepatocytes might be exposed to relatively high concentrations of a potential inhibitor dissolved in the intestinal lumen and the portal venous blood, respectively, compared to the concentrations in the systemic circulation. The OATP substrate pravastatin preferentially inhibited OATP1B1-mediated mesalazine uptake, but had also a significant effect on OATP1B3 and OATP2B1 transport function at the higher 100 μM concentration. The macrolide antibiotic clarithromycin significantly inhibited OATP1B1- and OATP1B3-mediated mesalazine uptake at the higher concentration. The OATP inhibitors cyclosporin A and rifampin had a more pronounced effect on mesalazine transport by OATP1B1 and OATP1B3 compared to OATP2B1. Azathioprine had no relevant effect on OATP function, whereas budesonide significantly affected mesalazine transport by all OATPs. These in vitro data indicate that local mesalazine concentrations and thus its effects could be reduced by some, concomitantly administered drugs in vivo. However, it should be noted that we do not have reliable measured values of inhibitor concentrations in the gut lumen or the portal vein making it very difficult to reliably predict the clinical consequences of these in vitro data.

Taken together, we identify here the molecular mechanisms of mesalazine uptake into intestinal cells and hepatocytes. Moreover, our data indicate that some comedications and genetic factors may modify intracellular mesalazine accumulation. Variability in mesalazine cellular uptake is likely to explain a fraction of the large interindividual variability in systemic plasma and local tissue concentrations of mesalazine. Since Wojtal et al. (Wojtal,
et al., 2009) recently showed a significant upregulation of SLCO2B1 mRNA in inflamed vs non-inflamed colon in patients with ulcerative colitis, we postulate that an increased intracellular accumulation of mesalazine contributes to the beneficial effects of this drug in the treatment of ulcerative colitis. Further studies are required to assess in greater detail the expression and localization of OATP proteins in healthy colon and in patients with inflammatory bowel disease.

Authorship Contributions

Participated in research design: Konig, Klotz, Fromm

Conducted Experiments: Konig, Glaeser, Mandery

Contributed new reagents or analytic tools: Keiser

Performed data analysis: Konig, Glaeser, Mandery, Fromm

Wrote or contributed to the writing of the manuscript: Konig, Glaeser, Keiser, Mandery, Klotz, Fromm

Other: NA
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Legends for figures

Fig. 1. Inhibition of OATP1A2-, OATP1B1-, OATP1B3- and OATP2B1-mediated BSP uptake by mesalazine. Inhibitory effects of mesalazine on OATP-mediated BSP (0.05 μM for OATP1B1, 1 μM for OATP1A2, OATP1B3 and OATP2B1) uptake were investigated after 10 min incubation. Data are shown as the percentage of the BSP uptake in the absence of mesalazine. Each value is the mean value ± standard deviation. ** p < 0.01 vs control.

Fig. 2. OATP1B1-, OATP1B3- and OATP2B1-mediated uptake of mesalazine (20 μM) in HEK-OATP1B1, HEK-OATP1B3 and HEK-OATP2B1 cells and the respective vector control cells. ** p < 0.01 vs control.

Fig. 3. Kinetics of OATP1B1-, OATP1B3- and OATP2B1-mediated net uptake of mesalazine. Net uptake was calculated as the difference between total uptake into HEK-OATP1B1, HEK-OATP1B3 and HEK-OATP2B1 cells and the respective vector control cells.

Fig. 4. Impact of the SLCO1B1 genetic variants *1b, *5 and *15 compared to the wild-type (*1a) on OATP1B1-mediated mesalazine net uptake. Net uptake was calculated as the difference between total uptake in the different HEK-OATP1B1 cells and the respective control cells. ** p < 0.01 wild-type (*1a) vs *1b, *5 and *15.

Fig. 5. Inhibition of the OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine (20 μM) uptake by different drugs. The inhibitory effect on OATP1B1-, OATP1B3- and OATP2B1-mediated mesalazine uptake was investigated using 10 μM and 100 μM of the
potential inhibitors. Data are shown as the percentage of the transporter-mediated mesalazine uptake in the absence of potential inhibitors (Co). Each value is the mean value ± standard deviation. Prava pravastain; Aza azathioprine; Clari clarithromycin; Bude budesonide; CsA cyclosporin A; Rifa rifampin; * P < 0.05; ** P < 0.01 vs control.
Table 1: Kinetic parameters of mesalazine uptake by *SLCO1B1*1a and its variants *SLCO1B1*1b, *SLCO1B1*5 and *SLCO1B1*15

<table>
<thead>
<tr>
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<th>$K_m$ (µM)</th>
<th>$V_{max}$ (pmol x mg Protein$^{-1}$ x min$^{-1}$)</th>
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<tr>
<td><em>SLCO1B1</em>1a</td>
<td>55.1 ± 13.4</td>
<td>28.3 ± 2.0</td>
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<tr>
<td><em>SLCO1B1</em>1b</td>
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<td><em>SLCO1B1</em>15</td>
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Fig. 1
Fig. 2
Fig. 3

**OATP1B1**

- $K_m = 55.1 \pm 13.4 \mu M$
- $V_{max} = 28.3 \pm 2.0 \text{ pmol} \times \text{mg Protein}^{-1} \times \text{min}^{-1}$

**OATP1B3**

- $K_m = 77.4 \pm 8.1 \mu M$
- $V_{max} = 85.1 \pm 3.1 \text{ pmol} \times \text{mg Protein}^{-1} \times \text{min}^{-1}$

**OATP2B1**

- $K_m = 188.9 \pm 40.5 \mu M$
- $V_{max} = 37.2 \pm 3.6 \text{ pmol} \times \text{mg Protein}^{-1} \times \text{min}^{-1}$
**Fig. 4**

Concentration mesalazine (µM) vs. Uptake (pmol x mg Protein⁻¹ x min⁻¹)
Fig. 5