Influence of Cyclooxygenase Inhibitors on the Function of the Prostaglandin Transporter Organic Anion-Transporting Polypeptide 2A1 Expressed in Human Gastroduodenal Mucosa

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ABSTRACT

The human organic anion-transporting polypeptide 2A1 (OATP2A1) is a prostaglandin transporter expressed in several tissues and plays an important role for local distribution of prostaglandins, which contribute to the integrity of gastric mucosa. Blockade of prostaglandin pathways by cyclooxygenase (COX) inhibitors has been associated with serious side effects such as gastrointestinal ulceration and bleeding. However, little is known regarding OATP2A1 expression in the upper gastrointestinal tract and the potential impact of cyclooxygenase inhibitors on OATP2A1 function. We first investigated the expression of OATP2A1 mRNA and protein in human gastroduodenal mucosa using human biopsy specimens obtained from antrum, corpus, and duodenum. The results indicate that OATP2A1 is expressed in the neck region and deep pyloric glands of antrum and in parietal cells of gastric corpus. Second, we examined various COX inhibitors for their effects on OATP2A1 transporter activity. Using HEK293 cells expressing OATP2A1, we found that diclofenac and lumiracoxib are potent inhibitors of OATP2A1-mediated transport of prostaglandin (PG) E2 with IC50 values of 6.2 ± 1.2 and 3.1 ± 1.2 μM. In contrast, indomethacin, ketoprofen, and naproxen led to significant stimulation of OATP2A1-mediated PGE2 transport by 162.7 ± 13.9, 77.2 ± 3.6, and 32.3 ± 4.9%, respectively. Taken together, our results suggest that various clinically used COX inhibitors have differential impact on the function of the prostaglandin transporter OATP2A1 in human stomach and that these effects may contribute to differences in the gastrointestinal side effects of COX inhibitors.

The SLCO (solute carrier organic anion transporter) gene family encodes for the organic anion-transporting polypeptides (OATPs) mediating uptake of various drugs and endogenous compounds (Hagenbuch and Meier, 2003, 2004; König et al., 2006). The rodent prostaglandin transporter Oatp2a1 was first identified in rats in 1995 (Kanai et al., 1995). The human prostaglandin transporter OATP2A1 was cloned from an adult kidney cDNA library and found to be expressed in several tissues such as lung, kidney, and intestine (Lu et al., 1996). Both human OATP2A1 and rat Oatp2a1 were found to transport various prostaglandins including PGE1, PGE2, PGD2, and PGF2α (Kanai et al., 1995; Lu et al., 1996). It was suggested previously that OATP2A1 might be involved in the termination of PGE2 effects by mediating the cellular uptake and the subsequent oxidation of prostaglandins in cytoplasm (Pitt et al., 1983; Schuster, 2002). In contrast, indomethacin, ketoprofen, and naproxen led to significant stimulation of OATP2A1-mediated PGE2 transport by 162.7 ± 13.9, 77.2 ± 3.6, and 32.3 ± 4.9%, respectively. Taken together, our results suggest that various clinically used COX inhibitors have differential impact on the function of the prostaglandin transporter OATP2A1 in human stomach and that these effects may contribute to differences in the gastrointestinal side effects of COX inhibitors.

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ABBREVIATIONS: SLCO, solute carrier organic anion transporter; OATP, organic anion-transporting polypeptide; PG, prostaglandin; NSAID, nonsteroidal anti-inflammatory drug; COX, cyclooxygenase; HEK, human embryonic kidney; PCR, polymerase chain reaction; VC, vector control; IRS, immunohistochemical rating score; ANOVA, analysis of variance; ASA, acetylsalicylic acid.
The nonsteroidal anti-inflammatory drugs (NSAIDs) are inhibitors of COX-1 and COX-2. The decrease in prostaglandin and thromboxane production leads to the desired analgesic, antipyretic, and anti-inflammatory therapeutic effects. On the other hand, these drugs frequently cause mucosal damage such as gastrointestinal erosions, ulceration, and severe bleeding episodes. Given that the integrity of gastroduodenal mucosa is highly dependent on the effects of locally synthesized prostaglandins (Gudis and Sakamoto, 2005), we raised the questions whether and where the prostaglandin transporter OATP2A1 is expressed in human gastroduodenal mucosa. Furthermore, we studied whether clinically used COX inhibitors influence the transport activity of OATP2A1, potentially contributing to gastrointestinal side effects. In the present study, we report the differential expression of the human prostaglandin transporter OATP2A1 in human gastroduodenal mucosa and the fact that different COX inhibitors are capable of inhibiting or stimulating the OATP2A1-mediated transport of PGE2.

Materials and Methods

Chemicals. [3H]PGE2 (185.6 Ci/mmol) was obtained from PerkinElmer Life Sciences GmbH (Rodgau-Jügesheim, Germany). Unlabeled PGE2 was purchased from Cayman Chemicals (Tattnall, Estonia), and poly-D-lysine hydrobromide was from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Sodium butyrate was purchased from Merck KGaA (Darmstadt, Germany). Acetylsalicylic acid, salicylic acid, diclofenac, ibuprofen, indomethacin, ketoprofen, and naproxen were dissolved in dimethyl sulfoxide. Lumiracoxib and salicylic acid were dissolved in ethanol. All other chemicals and reagents, unless stated otherwise, were obtained from Carl Roth GmbH + Co. KG (Karlsruhe, Germany) and were of the highest grade available.

Cell Culture and Generation of a HEK293 Cell Line Stably Expressing OATP2A1. HEK293 cells were cultured in minimal essential medium containing 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37°C and 5% CO2. The cells were routinely subcultured by trypsinization using trypsin (0.05%)-EDTA (0.02%) solution. All cell culture media supplements were obtained from Invitrogen GmbH (Karlruhe, Germany). The OATP2A1 expression plasmid was prepared by subcloning the SLC02A1 reference sequence (NM_000563.0) from the original pcCMV-SPORT6-OATP2A1 plasmid (purchased from Invitrogen GmbH) into the pcDNA3.1(-) vector (Invitrogen GmbH). HEK293 cells were transfected with the plasmid pcDNA3.1(-)-OATP2A1 using an Effectene transfection reagent kit (Qiagen, Hilden, Germany). After Geneticin (G418; 800 µg/ml) treatment, single colonies were selected and characterized for OATP2A1 mRNA and protein expression using real-time PCR, immunofluorescence, and immunoblot analyses. HEK293-VC cells were established by the same method using the plasmid lacking the insert for transfection.

Real-Time PCR. OATP2A1 mRNA expression was measured by quantitative real-time PCR using the LightCycler 2 System (Roche Diagnostics-Applied Science, Mannheim, Germany) and normalized to the housekeeping gene β-actin (expressed as arbitrary units). PCR was performed using Light Cycler FastStart DNA Master SYBR Green I reagents (Roche Diagnostics-Applied Science) and the following primers: OATP2A1 (forward 5′-GTGCGTGACCCAGGGAAGAAG-3′ and reverse 5′-GGAGGGTGTCGGGGAGG-3′; amplicon size of 588 base pairs), and β-actin (forward 5′-TGCGGCTCACACGACTTCG-CCATCTA-3′ and reverse 5′-CTGAGACATTCAGGCGGGATGGAGGG-3′; amplicon size of 661 base pairs). PCR fragments were amplified with an initial denaturation step of 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 10 s, annealing for 10 s at 64°C, and extending for 30 s at 72°C. After DNA amplification a melting curve analysis was performed.

Immunoblot Analysis. HEK293-OATP2A1 and HEK293-VC cells were seeded in poly-D-lysine-coated cell culture plates (diameter: 10 cm) at an initial density of 4.5 × 105 cells/plate. After 24 h, cells were treated with 10 mM sodium butyrate for additional 24 h before immunoblot analysis. Pelleted HEK293 cells were resuspended in protein storage buffer (100 mM Tris-HCl and 1 mM EDTA, pH 7.4) containing protease inhibitors (mini-complete protease inhibitor cocktail tablets; Roche Diagnostics-Applied Science, Mannheim, Germany). Protein concentrations were determined by the bicinchoninic acid assay (BCA Protein Assay Kit; Thermo Fisher Scientific, Waltham, MA). Five micrograms of total protein were diluted with Laemmli buffer (62 mM Tris-HCl, 2% SDS, 10% glycerol, 0.01% bromphenol blue, and 0.4 mM diithiothreitol) and incubated at 95°C for 5 min before separation on 10% SDS-polyacrylamide gels. A Benchmark PreStained Protein Ladder (Invitrogen GmbH) was used to visualize the protein molecular weight ranges. The proteins were transferred onto a nitrocellulose membrane (PROTRAN; Whatman Schleicher and Schuell, Dassel, Germany) using a tank blotting system from Bio-Rad Laboratories (Munich, Germany). The membrane was incubated with a polyclonal mouse anti-human OATP2A1 antibody (Abnova, Taipei City, Taiwan) or a mouse monoclonal anti-human β-actin antibody (Sigma-Aldrich Chemie GmbH). As a secondary antibody, horseradish peroxidase-labeled goat anti-mouse Fab fragments (Dianova, Hamburg, Germany) were used. HEK293 cells and HEK293-VC cells served as negative controls. Protein was visualized on autoradiography films (GE Healthcare UK Ltd., Little Chalfont, Buckinghamshire, UK) using ECL Western blotting detection reagents (GE Healthcare UK Ltd.) and a film developer (Kodak, Stuttgart, Germany). Films were scanned to perform a semi-quantitative analysis using Gel-Pro Analyzer software (version 4.5.00.0; Media Cybernetics Europe, Marlow, Buckinghamshire, UK). The cross-reactivity of the OATP2A1 antibody against other human OATPs was tested by performing an immunoblot analysis using homogenates of cells stably expressing OATP1A2, OATP1B1, OATP1B3, OATP2B1, OATP4A1, and OATP4C1. No cross-reactivity against the tested OATPs was observed.

Immunofluorescence Microscopy. HEK293-OATP2A1 and HEK293-VC cells were seeded on poly-D-lysine-coated coverslips placed in 12-well plates at an initial density of 5.5 × 105 cells/well. After 24 h, cells were treated with 10 mM sodium butyrate for 24 h. The immunofluorescence staining was performed according to a previously published protocol (Lee et al., 2005). The cells were washed with Tris-buffered saline and subsequently fixed in ice-cold 70% methanol for 10 min. Thereafter, the cells were permeabilized for 10 min using Tris-buffered saline-Triton X-100 (0.4%). The cells were blocked with 2% bovine serum albumin followed by incubation with a polyclonal mouse anti-human OATP2A1 antibody. A Cy2-conjugated AffiniPure goat anti-mouse IgG (Dianova) was used as secondary antibody. The nuclei were counterstained with SYTOX Orange dye (Invitrogen GmbH). The coverslips were inversely placed with an aqueous mounting medium (Thermo Fisher Scientific) on a microscope slide. The fluorescence was visualized using a confocal laser scanning microscope (Axiovert 100M; Carl Zeiss GmbH, Jena, Germany). The images were further processed using the Zeiss LSM Image Browser version 4.2.0.121 and Adobe Photoshop (CS2 version 9.0.2; Adobe Systems, San Jose, CA).

Transport Assays. The uptake experiments were performed as described previously (Seithel et al., 2007). In brief, HEK293-OATP2A1 and HEK293-VC cells were seeded in poly-D-lysine-coated 12-well plates at an initial density of 7.5 × 105 cells/well. After 24 h, cells were treated with sodium butyrate for 24 h before the uptake experiments. First, cells were washed with prewarmed (37°C) 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).
Subsequently, cells were incubated with a mix of radiolabeled and nonradiolabeled PGE₂ at 37°C for 5 min. For analyzing the inhibition of OATP2A1-mediated PGE₂ uptake, 0.5 μM PGE₂ was coincubated with different concentrations of the unselective COX inhibitors acetysalicylic acid, salicylic acid, diclofenac, ibuprofen, indomethacin, ketoprofen, and naproxen and the selective COX-2 inhibitor lumiracoxib at 37°C for 5 min. Afterward the cells were washed three times with ice-cold uptake buffer and lysed with 0.2% SDS. An aliquot was mixed with 4 ml of scintillation cocktail (Ultima Gold XR; PerkinElmer Life Sciences GmbH), and the intracellular accumulation of radioactivity was determined by liquid scintillation counting (TriCarb 2800; PerkinElmer Life Sciences GmbH). The appropriate protein concentration of each well was determined by bicinchoninic acid assay. All testing were performed at least in two separate experiments with a total of at least six wells per concentration studied.

**Immunohistochemical Analysis.** Paraffin-embedded biopsy samples from healthy subjects as well as macroscopically and microscopically judged healthy stomach sections of corpus, antrum, and duodenum were taken from the tissue archives of the Institute of Pathology (Friedrich-Alexander-University Erlangen-Nuremberg) following local ethical guidelines. Ten different samples from each region (antrum, corpus, and duodenum) were investigated. The immunohistochemical analysis was performed as described previously (Glaeser et al., 2007). In brief, paraffin sections were deparaffinized with xylene (Merck KGaA). After a rinse with ethanol, an antigen retrieval procedure was performed using citrate buffer, pH 6.0 (DCS GmbH and Co. KG, Hamburg, Germany). Afterward the sections were incubated with 10% H₂O₂ in Tris (pH 7.4, 100 mM) for 20 min at room temperature to quench endogenous peroxidase activity. After incubation of the sections with the blocking buffer Power Block Universal Blocking Reagent (BioGenex, San Ramon, CA) for 60 min, tissue sections were incubated overnight with a polyclonal mouse anti-human OATP2A1 antibody. Slides were subsequently incubated with biotinylated goat anti-mouse immunoglobulin and peroxidase-conjugated streptavidin (BioGenex). The proteins were visualized through a color reaction using 3-amino-9-ethylcarbazole or 3,3′-diaminobenzidine (AEC One Step Solution and Liquid DAB Substrate Pack; BioGenex) as substrates. Tissue sections were counterstained with Mayer's hematoxylin (BioGenex). The expression of OATP2A1 in antrum, corpus, and duodenum was graded using an immunohistochemical rating score (IRS) according to Remmele and Stegner (1987). In brief, the intensity was rated as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. The amount of positive stained cells was rated as follows: 0, no cells; 1, <10%; 2, 10–50%; 3, 51–80%; and 4, >80%. For the calculation of IRS, the scores of intensity and amount of positive cells were multiplied. Sporadic occurrence of positively stained edges of the tissue section was judged as an artificial effect and was therefore excluded from the IRS analysis. The specificity of immunoreactive signals for OATP2A1 was verified by negative controls, which were incubated with blocking buffer or polyclonal antisera that had been neutralized by preincubation with the antigenic peptide (Abnova) at 37°C for 1 h.

**Analysis of OATP2A1 Expression in Human Gastrodudodenal Biopsy Samples.** Samples for the investigation of mRNA and protein expression were obtained from previously published study (Venerito et al., 2006; Wex et al., 2008). In brief, healthy *Helicobacter pylori*-negative volunteers (n = 9) underwent a gastroduodenoscopy without any drug treatment. The biopsy samples from antrum, corpus, and duodenum were used to determine mRNA and protein expression of OATP2A1 as described above. All subjects gave written informed consent. The local ethics committees of the Otto-von-Guericke-University (Magdeburg, Germany) and Friedrich-Alexander-University (Erlangen-Nuremberg, Germany) approved the study.

**Data and Statistical Analysis.** The percentage of uptake inhibition or stimulation was calculated from control experiments in the absence of inhibitors or stimulators (100% uptake). The corresponding IC₅₀ or EC₅₀ values for inhibition or stimulation were calculated by fitting the data to a sigmoid dose-response regression curve (Prism 4.01 2004; GraphPad Software, San Diego, CA). The IC₅₀ or EC₅₀ value is the concentration at which 50% inhibition or stimulation of substrate uptake is obtained. All data are presented as means ± S.E.M. unless stated otherwise. The expression of OATP2A1 mRNA and protein in antrum, corpus, and duodenum was analyzed using one-way analysis of variance (ANOVA) with a Bonferroni multiple comparison test. The influence of COX inhibitors at a concentration of 100 μM on the OATP2A1 transport was analyzed using one-way ANOVA with Dunnett's multiple comparison test. p ≤ 0.05 was considered statistically significant.

**Results**

**Expression of OATP2A1 in Human Stomach.** First, we investigated the mRNA and protein expression of OATP2A1 in antrum, corpus, and duodenum of nine healthy subjects without any medications. OATP2A1 expression was normalized to that of β-actin. OATP2A1 mRNA expression was highest in antrum, and the difference between the antrum and the duodenum was found to be statistically significant (3.3-fold; p < 0.001, one-way ANOVA, Bonferroni multiple comparison test) (Fig. 1A). Interindividual variability of OATP2A1 mRNA expression among the nine volunteers was highest in antrum (8.5-fold) compared with corpus (6.4-fold) and duodenum (3.9-fold), respectively. However, OATP2A1 protein expression did not show statistical significance among antrum, corpus, and duodenum (p > 0.05, one-way ANOVA, Bonferroni multiple comparison test) (Fig. 1B). Figure 1C shows a representative immunoblot of OATP2A1 in biopsy samples obtained from human antrum, corpus, and duodenum.

**Localization of OATP2A1 in Human Stomach.** After our immunoblotting analysis indicating OATP2A1 expression in human gastroduodenal mucosa, we performed immunohistochemical staining to investigate the localization of OATP2A1. The results showed the most intense staining of OATP2A1 in the antrum mucosa (n = 10). Positive cytoplasmic and granular staining was found in the neck region and deep pyloric glands of antrum (Fig. 2A). Human corpus mucosa (n = 10) showed a cytoplasmatic granular staining also within the neck region, predominantly in parietal cells. In antrum and corpus, a possible luminal staining along the deep glands could be observed (Fig. 2, A and B). In duodenum (n = 10) several cells from the surface to the crypts in the mucosa and Brunner's glands showed positive staining for OATP2A1 (Fig. 2C). The IRS revealed values (mean ± S.D.) of 6.7 ± 3.2 for antrum, 4.3 ± 2.6 for corpus, and 1.9 ± 1.8 for duodenum. The positive staining in the parietal cells was substantially attenuated by preincubation of the OATP2A1 antibody with the antigenic peptide, indicating a specific binding of the antibody (Fig. 2, D and E).

**Characterization of HEK293-OATP2A1 Cell Line Stably Expressing OATP2A1.** To investigate the influence of nonselective and selective cyclooxygenase inhibitors on OATP2A1 function, we established a cell line stably expressing OATP2A1. Using real-time PCR and immunoblotting analysis, we confirmed that the selected clone of the HEK293-OATP2A1 cell line has elevated levels of OATP2A1 mRNA and protein expression compared with the HEK293-VC cell line (Fig. 3, A and B). The HEK293-OATP2A1 cells showed specific signals around 60 and 80 kDa, which could not be detected in the HEK293-VC cells and HEK293 parental cells. We further in-
Investigated the cellular localization of OATP2A1 using confocal microscopy. OATP2A1 was localized in cytoplasm and on the plasma membrane of cells (Fig. 3C). OATP2A1 expressed in HEK293 cells was functionally active as an uptake transporter of PGE2. HEK293-OATP2A1 cells were able to mediate the uptake of PGE2 (0.5 μM) with an uptake ratio of 17.4 compared with HEK293-VC cells (p < 0.001). Figure 3D shows the uptake of 0.5 μM PGE2 at 5 min into the HEK293-OATP2A1 and HEK293-VC cells.

Screening of COX Inhibitors for Influence on the OATP2A1 Function. To investigate the potential influence of COX inhibitors on the function of OATP2A1, we screened commonly used NSAIDs such as acetylsalicylic acid (ASA), salicylic acid, indomethacin, diclofenac, ibuprofen, ketoprofen, and naproxen and the selective COX-2 inhibitor lumiracoxib. ASA and salicylic acid (100 μM) showed significant inhibition of the PGE2 transport by 18 and 27% (p < 0.01). Diclofenac and lumiracoxib were found to be potent inhibitors, reducing the PGE2 transport by >95% (p < 0.01). In contrast, indomethacin, ibuprofen, ketoprofen, and naproxen showed a statistically significant stimulation of PGE2 transport at the tested concentration of 100 μM (p < 0.01) (Fig. 4).

Inhibition of OATP2A1-Mediated PGE2 Transport by Diclofenac and Lumiracoxib. After the initial screening shown in Fig. 4, we investigated the impact of varying diclofenac and lumiracoxib concentrations on the PGE2 transport (Fig. 5). The IC50 values of diclofenac and lumiracoxib for inhibition of OATP2A1-mediated PGE2 uptake were 6.2 ± 1.2 and 3.1 ± 1.2 μM, respectively.

Stimulation of OATP2A1-Mediated PGE2 Transport by Indomethacin, Ketoprofen, and Naproxen. Based on the results from the initial screening (Fig. 4), we further investigated the influence of varying concentrations of indomethacin, ibuprofen, ketoprofen, and naproxen on OATP2A1-mediated PGE2 transport. As shown in Fig. 6, the sigmoidal curve fitting revealed that indomethacin, ketoprofen, and naproxen showed a concentration-dependent stimulation of PGE2 transport by 162.7 ± 13.9, 77.2 ± 3.6, and 32.3 ± 4.9%, respectively. The EC50 value for indomethacin was 1.1 ± 1.4 μM. Ibuprofen did not show a dose-dependent stimulation or inhibition of OATP2A1-mediated PGE2 transport (data not shown).

Discussion

Here, we report that the prostaglandin transporter OATP2A1 is expressed in human gastroduodenal mucosa and that its transporter function can be inhibited or stimulated by commonly used COX inhibitors. Our study is the first detailed investigation of OATP2A1 expression in various regions of human stomach and duodenum. Our findings regarding interindividual variability in OATP2A1 expression and the differential impact of commonly used COX inhibitors on OATP2A1 function may be important in obtaining a better understanding of the mechanisms leading to the gastrointestinal side effects associated with various COX inhibitors.

In the present study, we investigated OATP2A1 expression at mRNA and protein levels in human biopsy samples. The real-time PCR analysis indicated that OATP2A1 mRNA expression was highest in the antrum, followed by corpus and duodenal mucosa. The immunohistochemical staining and semiquantitative IRS analysis revealed similar findings in terms of OATP2A1 protein expression. The immunoblotting analysis also indicated the highest expression in samples obtained from the antrum compared with corpus and duodenal mucosa. Although most of these data were consistent, the OATP2A1 expression levels in the duodenal biopsies based on the immunoblotting analysis appeared to be higher than expected compared with the immunohistochemical staining. These observed differences may be due to variability of OATP2A1 expression in the duodenal mucosa among individuals and may also reflect regional expression differences within the duodenum. However, they could also be the result of possible differences during biopsy collection. Taken together, our results support the expression of OATP2A1 in the human gastric and duodenal mucosa and the potential importance of OATP2A1 in prostaglandin signaling.
Fig. 2. Localization of OATP2A1 in human gastroduodenal tissues. Intense staining of antrum mucosa (A) in the neck region and pyloric glands was observed. Staining occurred in parietal cells of corpus mucosa (B) within the neck region and possible luminal expression in deep glands. Staining of duodenum (C) with positive cells throughout the surface and crypts was observed. Preincubation without (D) or with (E) the antigenic peptide is shown. The signal was almost abolished after preincubation with the peptide (E). A, B, and C, original magnification, 100×; staining with AEC. D and E, original magnification, 200×; staining with 3,3′-diaminobenzidine.

Fig. 3. Characterization of the HEK293-OATP2A1 cell line stably expressing OATP2A1. A, the generated HEK293-OATP2A1 cell line showed elevated expression of OATP2A1 mRNA compared with HEK293 cells transfected with the empty vector. B, immunoblot of OATP2A1 in HEK293-OATP2A1, HEK293-VC, and HEK293 parental cells. At the molecular masses of approximately 60 and 80 kDa, a specific signal was detected in HEK293-OATP2A1 cells, which was not detectable in HEK293-VC and HEK293 parental cells. C, immunofluorescence analysis of HEK293-OATP2A1 cells (left) and HEK293-VC cells (right) using confocal microscopy. OATP2A1 was visualized using a Cy2-conjugated secondary antibody (green fluorescence). The localization of OATP2A1 is shown in the x-y, x-z, and y-z layers. OATP2A1 was localized in cytoplasm and on the plasma membrane of the HEK293-OATP2A1 cells, whereas no staining is detectable in the HEK293-VC cells. The nuclei were stained red (original magnification, 400× with 4-fold zoom). D, uptake of 0.5 μM PGE2 into HEK293-OATP2A1 and HEK293-VC cells. The HEK293-OATP2A1 cells showed a significantly higher uptake (17.4-fold) of PGE2 compared with HEK293-VC cells (***, p < 0.0001, paired t test with Welch's correction). All data are displayed as means ± S.E.M.
Of note, our immunohistochemical analysis showed the expression of OATP2A1 in parietal cells and gastric glands with a granular cytoplasmic staining pattern, suggesting a possible expression of OATP2A1 in cytoplasmic vesicles. It has been reported that COX-1 and COX-2 are localized in endoplasmic reticulum and canalicular membranes of parietal cells and gastric glands of human stomach under normal and certain pathological conditions (Jackson et al., 2000; Bhandari et al., 2005). These results are in line with the previous studies reporting that OATP2A1/Oatp2a1 is often expressed in the same cells where prostaglandin synthesis and oxidation take place (Bao et al., 2002; Nomura et al., 2005). The authors further proposed a model of prostaglandin metabolism in which the prostaglandin synthesis, transport, and degradation take place in a compartmentalized manner. In support of this model, Oatp2a1 expression in rat kidney was localized in cytoplasmic vesicles along different regions of the collecting duct and epithelial and interstitial cells in the inner and outer medulla (Bao et al., 2002).

Given the OATP2A1 expression in the gastroduodenal mucosa, we further investigated whether COX inhibitors influence the function of OATP2A1. We generated a HEK293 cell line stably expressing OATP2A1, which showed a significantly higher transport of PGE2 compared with that in control cells. For the initial screening, various commonly used COX inhibitors were examined for their impact on OATP2A1 function using the concentration of 100 \( \mu M \). This concentration was pharmacologically relevant, corresponding to the therapeutic plasma concentrations for most compounds except for indomethacin (1–3 \( \mu M \)), diclofenac (2–10 \( \mu M \)), and lumiracoxib (4–20 \( \mu M \)).

First, our results indicated that diclofenac and lumiracoxib are potent inhibitors of OATP2A1 activity with comparable IC\(_{50}\) values. These results are not surprising because the chemical structures of the two compounds are closely related. It should be noted that diclofenac and lumiracoxib have a relatively low risk of gastrointestinal complications (Henry et al., 1996). This reduced risk with diclofenac and lumiracoxib was associated with the preferential or complete inhibition of COX-2 at therapeutic doses, respectively (Tegeder et al., 1999; Brune, 2007). It remains to be further investigated whether the low
risk of gastrointestinal complications of these drugs may also be related to their inhibitory effects on OATP2A1 function. On the other hand, indomethacin, ketoprofen, and naproxen stimulated OATP2A1-mediated PGE$_2$ uptake in a concentration-dependent manner. Although the molecular mechanism underlying the stimulatory effect of these compounds on the OATP2A1 function is unknown, similar findings showing the stimulation of uptake transporter activities by drugs and endogenous substrates have been reported previously for transporters other than OATP2A1 (Grube et al., 2006; Bachmakov et al., 2008). Regarding OATP2A1, Lu et al. (1996) also observed a slight increase of 14% in OATP2A1-mediated PGE$_2$ transport at a concentration of 10 µM indomethacin, whereas at 100 µM indomethacin the PGE$_2$ uptake was decreased to 82.5% of control. This discrepancy with our study may be explained by differences in the total substrate concentration of PGE$_2$. Currently, it is not known whether the differential impact of COX inhibitors on OATP2A1 function may contribute to the gastrointestinal side effects of COX inhibitors. NSAID-induced gastroduodenal complications are most frequent in the antrum, followed by the corpus (Kamada et al., 2006), and our results indicate that OATP2A1 expression levels follow the same order. It is known that the blockade of cyclooxygenases by COX inhibitors is a major cause for NSAID-induced gastrointestinal ulcers. Our study indicates that some COX inhibitors may also modulate the activity of OATP2A1 in addition to the well known inhibition of cyclooxygenases, thus influencing the concentrations of prostaglandins in gastric mucosa. The role of OATP2A1 in NSAID-induced ulcerations and bleeding episodes remains to be further elucidated.

Furthermore, Brune et al. (1977) demonstrated that parietal cells of stomach are capable of trapping salicylates. This finding seems to be of special interest considering the localization of OATP2A1 in parietal cells in human stomach. Whether transport proteins such as OATPs mediate this trapping needs to be investigated.

In summary, we characterized the expression of the prostaglandin transporter OATP2A1 in human gastroduodenal mucosa, where it is predominantly expressed in antrum and corpus mucosa. Furthermore, we demonstrate that the activity of OATP2A1 is distinctly modulated by unselective and selective COX inhibitors, suggesting a potential pathophysiological role of OATP2A1 in the gastrointestinal side effects of COX inhibitors.

References
Remmele W and Stegner HE (1987) [Recommendation for uniform definition of an immunoreactive score (IRS) for immunohistochemical estrogen receptor detection (ER-ICA) in breast cancer tissue]. *Patholologie 8*:138–140.

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