The Prostaglandin Transporter OATP2A1 Is Expressed in Human Ocular Tissues and Transports the Antiglaucoma Prostaglandin Latanoprost

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PURPOSE. Latanoprost, a prostaglandin F₂α analogue, has become one of the most widely used medications for the treatment of glaucoma. The authors hypothesized that organic anion transporting polypeptides (OATPs) are responsible for the uptake of latanoprost into ocular tissues and, hence, that they contribute to the interindividual differences in drug concentrations and effects.

METHODS. Expression of prostaglandin (PG) transporters (OATP2A1, OATP2B1) in human ocular tissues was determined using real-time RT-PCR and immunofluorescence. The inhibitory interactions between latanoprost and its active metabolite (the free acid) and the uptake of prototypical substrates (PGE₂ and bromosulfophthalein) were tested in stably transfected human embryonic kidney cells overexpressing either OATP2A1 or OATP2B1. These cells were also used to investigate whether latanoprost and latanoprost acid are substrates of OATP2A1 or OATP2B1.

RESULTS. OATP2A1 and OATP2B1 mRNA expression was highest in the choroid/retinal pigment epithelium (RPE) complex and ciliary body. OATP2A1 protein expression was most prominent in the RPE and in epithelial and endothelial cell layers of anterior segment tissues, such as cornea, conjunctiva, iris, and ciliary body, whereas OATP2B1 protein was additionally expressed in trabecular meshwork, Schlemm canal, and choroidal vasculature. Latanoprost and latanoprost acid significantly inhibited both OATP2A1 and OATP2B1. Uptake experiments demonstrated that latanoprost acid is effectively transported by OATP2A1 (affinity constant \( K_a \), 5.4 M⁻¹; maximum uptake rate \( V_{\text{max}} \), 21.5 pmol/mg protein/min) and less effectively by OATP2B1.

CONCLUSIONS. The results presented herein suggest that at least OATP2A1 plays a role in the intraocular disposition of the therapeutically used prostaglandin latanoprost. (Invest Ophtalmol Vis Sci. 2010;51:2504–2511) DOI:10.1167/iovs.09-4290

The prostanooids are an established group of ocular hypotensive drugs used for the clinical management of glaucoma. The group includes five chemical compounds that are structurally derived from the naturally occurring prostaglandin (PG) F₂α, latanoprost, bimatoprost, travoprost, and unoprostone. Latanoprost is the most widely used antiglaucoma prostanooid. Prostanoids increase uveoscleral and trabecular (conventional) outflow through several mechanisms, including changing the cell shape, cytoskeletal alterations, and remodeling of the extracellular matrix.1,2,3

Topical application of the prodrug latanoprost (ester) leads to absorption of approximately 1% of the drug through the cornea, where it is completely hydrolyzed to the biologically active acid form.4,5 A previous study, as well as our own pilot experiments, demonstrated remarkable interindividual variations in the concentration of latanoprost acid in the aqueous humor after a single 1.5-μg dose of latanoprost,5 a finding that might be related to interindividual variations in the clearance of the drug. At physiological pH, prostanoids predominate as charged organic anions and diffuse poorly through the lipid bilayer of endothelial cells.6,7 Therefore, the clearance of prostanoids from the aqueous humor might be dependent on active uptake mechanisms.

We hypothesized that uptake transporters are involved in the clearance of latanoprost from the aqueous humor, and we focused on two transporters, both of which belong to the organic anion transporting polypeptide (OATP) family.8–11,12 OATP2A1 (gene name SLC20A1) and OATP2B1 (gene name SLC20B1). The functional profile of OATP2A1 expressed in vitro strongly suggests a role primarily in PGE₂ and PGF₂α uptake and degradation.12–15 Although OATP2A1 mRNA expression has been demonstrated in the human eye,16 its ocular expression pattern at the protein level has not been investigated. Moreover, it remains to be established whether the antiglaucoma prostanoids that are used clinically are readily transported by OATP2A1.

OATP2B1 is expressed at significant levels in the human ciliary body epithelium.17,18 Functionally, OATP2B1 has a relatively narrow substrate spectrum and transports, for example, bromosulfophthalaein (BSP), estrone-3-sulfate, and dehydroepiandrosterone sulfate. Although the findings regarding OATP2B1-mediated transport of endogenous PGs such as PGE₂ remain controversial,9,19–21 one study has demonstrated low-affinity transport of the PGF₂α-derived antiglaucoma drug unoprostone carbonate.17 Whether other antiglaucoma prostanoids are also transported by OATP2B1 remains unclear.

In this study we addressed whether OATP2A1 or OATP2B1 is involved in the cellular uptake of latanoprost and, therefore, in the ocular clearance of this widely used antiglaucoma drug.
**Materials and Methods**

**Aqueous Humor and Human Tissue Samples**

Twelve patients (nine women, three men; age range, 60–95 years) received one 30-μL drop of 0.005% latanoprost (Xalatan; Pfizer, New York, NY) into the eye. Solutions were administered 23 to 159 minutes before routine cataract surgery. Aqueous humor samples (50 μL) were withdrawn from treated eyes at surgery initiation. Times from instillation to sampling were recorded. Before entry into the study, the patients signed informed consent forms. The study was approved by the ethics committee of the University of Erlangen-Nuremberg.

Human whole globes, enucleated within 6 hours of death from human organ donors, were immediately dissected, and the following tissues were collected: choroida/retinal pigment epithelium (RPE), sensory retina, iris, ciliary body, lens, and cornea. The tissues were snap-frozen in liquid nitrogen and stored at −80°C until processing. All tissues were from donors free of ocular disease and other systemic complications. For comparison of transporter expression, human tissue samples from brain cortex (n = 2), liver (n = 3), placenta (n = 2), heart, and kidney (n = 3) were studied (tissues were frozen within 6 hours of sampling). These human biological materials were rendered anonymous by irreversibly removing identifiers, and their use was authorized using analysis software (Analyst 1.4.2; Applied Biosystems, Foster City, CA).

**LC/MS/MS Assay of Latanoprost Acid in Aqueous Humor Samples**

We added 50 μL internal standard solution (PGE₂-d₄, 10 ng/mL in methanol; Cayman, Ann Arbor, MI), 5 μL methanol, and 2 μL 3-butyln-methylether to 50-μL aqueous humor samples. After extraction for 15 minutes and then centrifugation, 1.5 mL of the organic layer was transferred into glass tubes and dried under a stream of nitrogen before reconstitution in 100 μL eluent plus 0.1% formic acid. The injected volume was 40 μL. HPLC was performed with an Agilent system (Series 1100; Agilent Technologies Deutschland GmbH, Böblingen, Germany). An HPLC column (Synergy 4-μm Hydro-RP 80 A 150 × 2.0 ID) with guard column (Phenomenex, Aschaffenburg, Germany) was used for chromatographic separation, a mixture of 40% acetonitrile (LC-MS grade), and 60% water (LC-MS grade) as the mobile phase. Flow rate was set at 0.5 mL/min; retention times were 2.5 minutes for latanoprost acid and 3.0 minutes for internal standard.

Quantification was performed with a triple quadrupole mass spectrometer (Sciex API 4000; Applied Biosystems, Toronto, ON, Canada) with a turbo ion spray interface using multiple reaction monitoring in the negative ion mode. The transmissions for latanoprost acid were 389.4 m/z (Q1; 355.0 m/z for internal standard) and 345.2 m/z (Q3; 237.0 m/z for internal standard), and main MS parameters were as follows: collision gas, 8; curtain gas, 25; nebulizer gas (GS 1), 30; turbo gas (GS 2), 10; ion spray voltage, −4000 V; and temperature, 500°C. The peak area ratio of latanoprost acid to internal standard was calculated using analysis software ( Analyst 1.4.2; Applied Biosystems, Foster City, CA).

The lower limit of quantification was 1 ng/mL. A calibration curve was constructed using 1/x-weighted linear regression between spiked aqueous humor concentrations and the measured ratios, which were linear over the range 1 to 50 ng/mL (r = 0.99995). Aqueous humor calibration standards (1, 2.5, 5, 10, 25, and 50 ng/mL) and quality controls, blank samples, and double-blank samples were prepared in the same manner. Quality controls were routinely assayed at 2500, 312.5, 59.1, and 4.9 μM. Quality control intraday coefficients of variation were 2.8%, 3.8%, 4.9%, and 6.2%, and interday coefficients of variation were 8.0%, 2.2%, 1.5%, and 3.3%.

**Quantitative Real-Time RT-PCR**

Total RNA from choroida, retina, iris, ciliary body, lens, and cornea (n = 5 each) was extracted with an RNA isolation kit (Illustra RNAspin Mini; GE Healthcare, Little Chalfont, Buckinghamshire, UK). Total RNA (500 ng each) was reverse-transcribed (Superscript III; Invitrogen, Carlsbad, CA), and cDNA was subsequently amplified (45 cycles of 50°C for 2 minutes, 95°C for 15 seconds, and 60°C for 1 minute) with a sequence detection system (ABI 7900HT; Applied Biosystems). Pre-designed primers and TaqMan probes for SLCO2A1 (OATP2A1), SLCO2B1 (OATP2B1), and the housekeeping genes ACTB (β-actin) and GAPDH (glyceraldehyde 3-phosphate dehydrogenase) were used (Hs00194554_m1, Hs00200670_m1, Hs00537333_g1, Hs00260705_g1; Applied Biosystems). Primers were designed to amplify DNA fragments crossing exon/exon boundaries. For comparison, OATP2A1 and OATP2B1 mRNA levels were determined in human liver, brain, kidney, placenta, and heart cDNA pools under equal conditions. Relative amounts of OATP2A1 and OATP2B1 mRNA were calculated using the ∆δelta-CT method22 by relating the PCR signal of the target transcript in human tissue samples to that in the choroid samples, which served as a calibrator. Target mRNA levels were normalized to the internal control genes ACTB and GAPDH according to the equation: relative target mRNA level = 2^-ΔΔCTTarget/Actb&GAPDH, where ΔδeltaCT = (CTTarget–CTagtAChoroid)–(CTTarget–CTagtBChoroid) and ΔΔCT = (ACTB&GAPDH)Tissue – (ACTB&GAPDH)Choroid.

**Immunofluorescence**

Five human donor eyes were investigated. Each eye was divided with a razor blade at the equator in an anterior and a posterior part. From each segment for each antibody, at least six sections were cut. PFA-fixed eyes from human organ donors were washed with phosphate-buffered saline (pH 7.4) and placed in embedding medium (Tissue-Tek OCT; Jung, Nussloch, Germany). Tissues were frozen at −20°C and serial sectioned into 12-μm thin slices with a cryotome (Kryosatm CM 3050S; Leica, Bensheim, Germany). Paraffin-embedded tissue was sectioned at 7 μm thin and was deparaffinized in xylol. Sections placed on poly-L-lysine-coated slides were incubated with dry milk solution (Blotto; Santa Cruz Biotechnology, Heidelberg, Germany) for 1 hour at room temperature to prevent nonspecific staining.

OATP2A1 polyclonal antibody was from Abnova (Heidelberg, Germany). OATP2B1 antiserum was raised in rabbits using a peptide corresponding to the carboxy-terminal amino acids 690 to 709 of human OATP2B1 (AVEQQLLSVGPGKPDGSRVS; NCBI accession number NP_099187) coupled to keyhole limpet hemocyanin (Peptide Specialty Laboratories, Heidelberg, Germany). The specificity of both antisera was confirmed (53,24). Slides were incubated with diluted (1:100) dilution in blocking buffer: 2% bovine serum albumin and 0.2% Triton X-100 in Tris-buffered saline) antibodies at 4°C overnight. After a wash in Tris-buffered saline, the sections were incubated with goat anti-mouse IgG Cy-2 or swine anti-rabbit IgG Cy-2 (1:100; Dianova, Hamburg, Germany) in blocking buffer for 2 hours at room temperature.

Stained sections were analyzed by confocal laser microscopy (MRC 600 confocal imaging system; Bio-Rad Laboratories, Richmond, CA) under a microscope (Axioplan; Zeiss, Oberkochen, Germany).

**Cell Lines Overexpressing OATP2A1 and OATP2B1**

Human embryonic kidney (HEK) 293 cells were stably transfected with the SLCO2A1 cDNA (GenBank ID, NM_005630) encoding human OATP2A1. The recombinant OATP2A1 protein was integrated into the plasma membrane of HEK-OATP2A1 cells and was functionally active, as demonstrated previously.23 The HEK-OATP2B1 cell line was established by stable transfection with the SLCO2B1 cDNA (isoform 1; GenBank ID, NM_007256). Transfections were carried out as described.23 For uptake experiments, the cells (500,000/well) were
seeded in 24-well plates coated with 0.1 mg/mL poly-D-lysine and grown to confluence for 2 days.

**Competition Assays**

For inhibition studies, the prototypical tracer substrates [3H]PGE₂ (Perkin Elmer, Boston, MA; 185.6 Ci/mmol) and [3H]-BSP (Hartmann Analytic, Braunschweig, Germany; 19.5 Ci/mmol) for OATP2A1 and OATP2B1 uptake, respectively, were used. Unlabeled compound (PGE₂ [Cayman, Ann Arbor, MI], BSP [Sigma-Aldrich, Taufkirchen, Germany]) was added to reach a final substrate concentration of 1 μM each. Before experiments were started, the 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). Cells were then incubated with tracer substrate in the presence or absence of latanoprost or latanoprost acid (1, 3, 10, 30, 100, 300, 1000 μM) at 37°C. It is well known from previous studies that OATP2A1-mediated uptake of PGE₂ is linear for up to 10 minutes. In contrast, time-dependent uptake of BSP in HEK-OATP2B1 cells was not previously investigated. Our own results demonstrated linear uptake for at least 10 minutes. To ensure sufficient baseline uptake of the tracer substrate in our inhibition experiments, an incubation period of 10 minutes was chosen. Subsequently, the cells were washed three times with ice-cold uptake buffer and lysed with 5 mM Tris-HCl (pH 7.5) plus 0.1% Triton X-100. The intracellular accumulation of tracer was measured by liquid scintillation counting (Perkin Elmer), and uptake was related to the concentration of cellular proteins, which was determined with bicinconic acid assay (BCA Protein Assay Kit; Thermo Scientific, Rockford, IL).

**Uptake Assay**

Cells (HEK-OATP2A1 or HEK-OATP2B1 and the respective vector controls) were incubated with uptake buffer containing either latanoprost or latanoprost acid at 37°C for the indicated periods. Subsequently, cells were washed three times with ice-cold uptake buffer before they were lysed with 5 mM Tris-HCl (pH 7.5) plus 0.1% Triton X-100. The intracellular accumulation of tracer was measured by liquid scintillation counting (Perkin Elmer), and uptake was related to the concentration of cellular proteins, which was determined with bicinconic acid assay (BCA Protein Assay Kit; Thermo Scientific, Rockford, IL).

**LC/MS/MS Assay for Latanoprost and Latanoprost Acid in Cell Lysates**

Ninety microliters of internal standard solution (latanoprost: etoricoxib [i.e., etoricoxib is the internal standard for latanoprost] 5 ng/mL in acetonitril/H₂O 40:60 [vol/vol] plus 0.1% formic acid; latanoprost acid: PGE₂-d₄ 10 ng/mL in methanol/H₂O 25:75 [vol/vol] plus 0.1% formic acid) were added to 10-μL cell lysate samples. Injected volume was 40 μL.

Quantification was performed as described for aqueous humor samples with the following modifications for latanoprost: multiple reaction monitoring was used in the positive ion mode, and another HPLC column (Nucleodur C18 5 μm 100 A 30 × 2.0 ID; Macherey-Nagel, Düren, Germany) was used for chromatographic separation. Retention times were 6.1 minute for latanoprost and 0.9 minute for the internal standard. Transmissions for latanoprost were 453.1 m/z (359.1 m/z for internal standard) for Q1 and 379.3 m/z (280.2 m/z for internal standard) for Q3. The primary MS parameters were as follows: collision gas, 4; curtain gas, 10; nebulizer gas (GS 1), 40; turbo gas (GS 2), 20; ion spray voltage, 4500 V; temperature, 700°C.

Calibration curves were constructed using 1/X-weighted linear regression (latanoprost) or 1/X²-weighted quadratic regression (latanoprost acid) between spiked cell lysate concentrations and the measured ratios, which were linear over the range 9.8 to 2500 ng/mL (latanoprost) and 1.2 to 2500 ng/mL (latanoprost acid).

**Data and Statistical Analyses**

Inhibition dose-response curves were analyzed by curve-fitting and calculation of IC₅₀ values with appropriate software (Prism; GraphPad, 2001).
Figure 3. Immunolocalization of OATP2A1 in human ocular tissues (green fluorescence, left) and corresponding phase-contrast images (right). Scale bar, 20 μm. (A) Epithelium (CEP) and stroma of the cornea (CS). (B) Endothelium (CEN) and stroma of the cornea (CS). (C) Conjunctival epithelium (CJE). (D) Stroma (IS), vessels, and pigmented epithelium (IPE) of the iris. (E) Ciliary body with its nonpigmented (CBNPE) and pigmented (CBPE) epithelium. (F) Choroid (CH) and RPE.

Figure 4. Immunolocalization of OATP2B1 in human anterior eye tissues (green fluorescence, left) and corresponding phase-contrast images (right). Scale bar, 20 μm. (A) Epithelium (CEP) and stroma of the cornea (CS). (B) Endothelium (CEN) and stroma of the cornea (CS). (C) Conjunctival epithelium (CJE). (D) Stroma (IS), vessels, and pigmented epithelium of the iris. (E) Ciliary body with its nonpigmented (CBNPE) and pigmented (CBPE) epithelium. (F) Choroid (CH) and RPE.
RESULTS
Latanoprost Acid Levels in the Aqueous Humor
Concentrations of the active drug, latanoprost acid, were determined at 23 to 159 (mean, 91) minutes after the topical application of a single 1.5-μg dose of latanoprost. For ethical reasons, multiple sampling was not possible (e.g., to generate time-concentration curves for each patient). Figure 1 shows the intraocular concentrations of latanoprost acid in patients as a function of time from latanoprost application. Based on these data, a nonlinear regression fit was calculated, representing the average course of latanoprost acid concentrations in the aqueous humor over time. Of note, we observed a marked deviation from the average concentration-time curve in some samples, suggesting a considerable interindividual variation of intraocular latanoprost concentrations.

OATP2A1 and OATP2B1 mRNA Expression in Ocular Tissues
Transcript levels of the uptake transporters OATP2A1 and OATP2B1 were determined in human ocular tissues by real-time RT-PCR. For comparison, expression levels were also investigated in human nonocular tissues, such as liver, brain, kidney, placenta, and heart. In these tissues, OATP2A1 and OATP2B1 transcript levels were highest in the placenta and the liver, respectively, which fits well with previous reports.

In ocular tissues, mRNA expression of both OATP2A1 and OATP2B1 was highest in the choroid/RPE-complex and in the ciliary body and was much lower in the sensory retina, iris, lens, and cornea (Fig. 2).

Ocular Localization of OATP2A1 and OATP2B1 Protein
Both OATP2A1 and OATP2B1 proteins were expressed in virtually all tissues of the human eye, showing a similar distribution pattern in anterior segment tissues but differential expression patterns in posterior segment tissues, such as retina and choroid. OATP2A1 immunoreactivity was detected in the corneal epithelium, particularly its superficial layers, in the corneal endothelium, and in stromal keratocytes (Figs. 3A, 3B). Intense staining could also be observed in the suprabasal epithelial cells of the bulbar conjunctiva (Fig. 3C). Expression of OATP2A1 was apparent in the vessels of the iris stroma, both in vascular endothelial and adventitial cells (Fig. 3D). Moreover, OATP2A1 could be immunolocalized to the basal aspects of the nonpigmented and pigmented epithelium of the ciliary body facing the aqueous humor and ciliary stroma, respectively (Fig. 3E). In the posterior part of the human eye, only weak staining was observed in the RPE, whereas the sensory retina and the choroid were essentially negative (Fig. 3F).

Immunolocalization of OATP2B1 showed staining patterns in anterior segment tissues similar to those of OATP2A1 (i.e., in basal and superficial cells of the corneal and conjunctival epithelia; Figs. 4A, 4C), the corneal endothelium (Fig. 4B), vascular and perivascular cells of the iris and ciliary stroma (Figs. 4D, 4E), basal aspects of the ciliary epithelia (Fig. 4E), and endothelial cells of the trabecular meshwork and Schlemm canal. However, strong immunofluorescence for OATP2B1 was observed not only in the RPE (Fig. 4F) but also in the vascular endothelia of choroidal vessels (Fig. 4F).

OATP2A1- and OATP2B1-Dependent Transport
HEK cells stably overexpressing OATP2A1 or OATP2B1 were generated so that the significance of these transporters could be studied in the uptake of prostanoid antiglaucoma drugs. Figure 5 shows the concentration-dependent effects of latanoprost and latanoprost acid on OATP2A1- and OATP2B1-mediated uptake of their respective prototype substrates. Both latanoprost and latanoprost acid were potent inhibitors of OATP2A1-mediated uptake of PGE₂, with IC₅₀ values in the low micromolar range (latanoprost acid IC₅₀, 0.7 μM [95% CI, 0.6–0.8 μM]; latanoprost acid IC₅₀, 3.2 μM [95% CI, 2.8–3.7 μM]).

Prostanoids were less potent inhibitors of OATP2B1-mediated BSP uptake (latanoprost acid IC₅₀, 23 μM [95% CI, 15–38 μM]; latanoprost acid IC₅₀, 88 μM [95% CI, 59–130 μM]).
Figures 6 and 7 demonstrate that, when expressed in HEK cells, OATP2A1 and OATP2B1 mediate the rapid, time-dependent uptake of latanoprost acid. For both OATP transporters, intracellular accumulation of latanoprost acid, when applied at a concentration of 50 \( \mu \text{M} \), reached equilibrium by 5 minutes. To evaluate the binding characteristics (affinity constant, \( K_m \)) and the maximum uptake rates (\( V_{\text{max}} \)) of the OATP transporters, the initial uptake (4 minutes’ incubation) of latanoprost acid at different concentrations was investigated. In HEK-OATP2A1 cells, net uptake was saturable with \( K_m = 5.4 \ \mu \text{M} \) and \( V_{\text{max}} = 21.5 \ \text{pmol/mg/min} \) (Figs. 6B, 6C). In contrast, OATP2B1-mediated net uptake of latanoprost acid was not saturable up to 125 \( \mu \text{M} \) latanoprost acid. Nevertheless, the uptake of latanoprost acid at 15 \( \mu \text{M} \) was twofold higher in HEK-OATP2B1 compared with control cells (Fig. 7B). Altogether, the results indicate that latanoprost acid is readily transported by OATP2A1 but is a weak substrate of OATP2B1.

We also investigated whether latanoprost (the ester prodrug) is transported by OATP2A1 or OATP2B1. Even when the compound was added to the cells at the highest concentration (500 \( \mu \text{M} \)), neither latanoprost nor its metabolite, latanoprost acid, was detected within the cells by LC/MS/MS. Taken together, the results suggest that the active acidic metabolite of latanoprost, but not the ester prodrug, is the substrate of the uptake transporters OATP2A1 and OATP2B1.

### DISCUSSION

More than three decades ago, Bito et al.\(^{27-29}\) provided the first evidence that the facilitated removal of PGs from the eye occurs by way of an active transport mechanism. Twenty years later, the molecular mechanisms underlying PG transport were unraveled by cloning the cDNA of potential PG transporters, such as OATP2A1.\(^{12,26}\) Here we demonstrate for the first time that latanoprost, the most widely used PG for the treatment of elevated intraocular pressure in glaucoma, is a high-affinity substrate of OATP2A1 that is expressed predominantly in the choroid/RPE-complex and in the ciliary body of the human eye.

The active metabolites of antiglaucoma drugs are negatively charged under physiological pH, and it is generally believed that they cannot cross cell membranes without an active transportation system. Previous work proposes members of the OATP/SLC family—OATP1A2, OATP2B1, OATP4A1—as candidates for uptake of the active carboxylic (M1) metabolite of unoprostone.\(^{17}\) Among these transporters, unoprostone M1 had the highest affinity to OATP2B1, followed by OATP1A2 and OATP4A1. Our results extend this previous observation by the demonstration that latanoprost acid is also a substrate of OATP2B1. Moreover, we have demonstrated the expression of OATP2B1 in the choroid/RPE-complex and ciliary body at relevant levels, both on the mRNA level and protein level,
whereas expression in other eye tissues was markedly lower (sensory retina, iris, lens, cornea). The rank-order expression levels of OATP2B1 in ocular tissues largely conform to recently reported data from Zhang et al. who investigated three major compartments of the human eye. In that study, the relative expression of OATP2B1 was higher in the iris/ciliary body than in the retina/choroid or cornea.

Although several studies have implicated OATP family members other than OATP2A1 in PG transport in the human eye, few have focused on the role of OATP2A1 for the ocular disposition of PGs. In their pioneering work, Schuster et al. demonstrated that OATP2A1 mRNA is expressed in a broad variety of human ocular tissues, though expression levels were not quantified and compared with those of human nonocular tissues. In the present study, we show that OATP2A1 mRNA is relatively abundant in the choroid/RPE-complex and ciliary body compared with other tissues, such as liver, brain, kidney, and heart. On the protein level, OATP2A1 expression was most prominent in epithelial and endothelial cell layers of anterior segment tissues, whereas expression in the posterior segment was weak. In contrast, OATP2B1 mRNA and protein were detected primarily in the ciliary body and choroid/RPE complex and could also be localized to epithelial and endothelial cell layers, particularly vascular endothelia of the choroidal stroma. Protein expression of this transporter in blood vessels has been reported previously. Furthermore, the protein expression detected in our study largely conforms to the investigations of OATP2B1 in the human ciliary body, demonstrating expression at the inner border of the epithelium facing the aqueous humor. In addition, OATP2B1 expression was observed in the trabecular meshwork and Schlemm’s canal.

Unlike most other organs, the eye has no PG-metabolizing enzymes. Therefore, a physiological role of OATP2A1 could be the clearance of endogenous PGs synthesized in the ciliary epithelium and the ciliary body. In addition, OATP2A1 may contribute to the clearance of antiglaucoma prostanooids from the aqueous humor. Indeed, we have demonstrated that latanoprost acid is a substrate of OATP2A1 with a relatively higher affinity than for OATP2B1.

In summary, the present study confirms the considerable variability in concentrations of the biologically active form of latanoprost in the aqueous humor after topical application of a standard dose of the drug. Protein expression of OATP2A1 and OATP2B1 was demonstrated in virtually all tissues of the anterior and posterior segments of the human eye. Moreover, we have provided experimental evidence that transporters from the OATP family, particularly OATP2A1, are involved in the clearance of latanoprost acid from the aqueous humor and may thereby influence the bioavailability of latanoprost in patients.

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References