Supplementary Material

Significance of organic anion transporter 2 and organic cation transporter 2 in creatinine clearance: Mechanistic evaluation using freshly-prepared human primary renal proximal tubule cells

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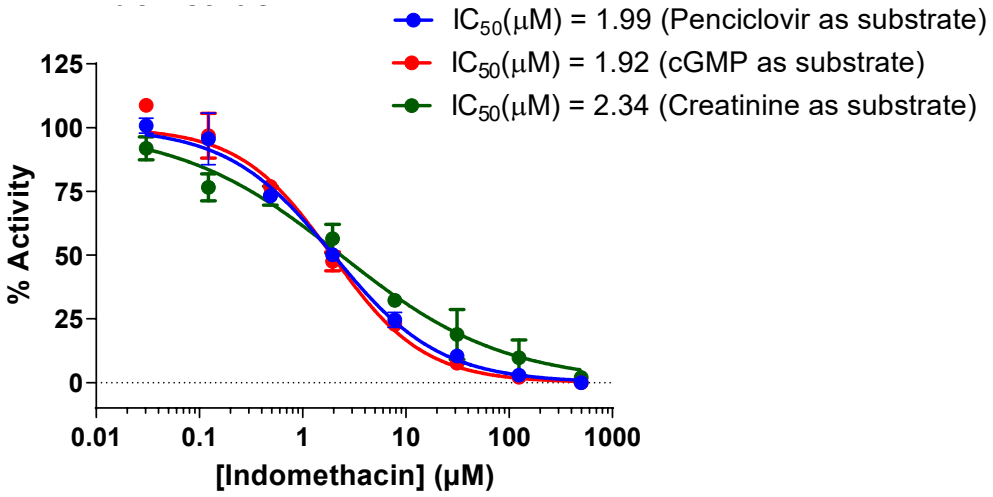

Supplementary Figure 1. Inhibition of OAT2-mediated creatinine, penciclovir and cGMP uptake by indomethacin in transfected HEK293 cells. Datapoints represent mean ± S.D. (n=3).

Supplementary Table 1. Uptake ratios of creatinine, metformin and penciclovir in transfected-HEK293 cells.

	^{14}C-Creatinine **(10 µM)**	^{14}C-Metformin **(10 µM)**	^{3}H-Penciclovir **(1 µM)**
Transporters	Uptake ratio (mean ± s.d., n=3)*		
OAT1	0.55 ± 0.05	0.83 ± 0.13	0.89 ± 0.10
OAT2	7.36 ± 0.88	1.6 ± 0.08	23.03 ± 1.39
OAT3	0.81 ± 0.14	1.04 ± 0.13	1.65 ± 0.14
OCT2	28.14 ± 4.46	39.21 ± 0.71	1.03 ± 0.02
MATE1	20.49 ± 0.94	22.15 ± 0.22	1.66 ± 0.08
MATE2K	7.72 ± 0.21	7.8 ± 0.55	1.06 ± 0.01

*Uptake ratio is ratio of cell accumulation in transfect- to wild-type cells, measured at 2 min.

Supplementary Methods

Chemicals and Reagents

All the compounds used in the assay were obtained from Pfizer chemical inventory system or procured from Sigma-Aldrich (St.Louis, MO). HEK293 cells transfected with OCT2 were obtained from Dr. Kathleen Giacomini (UCSF, CA). HEK293 cells transfected with OAT2-variant 1 were obtained from Dr. Ryan Pelis (Halifex, Canada). HEK293 cells transfected with MATE1/2K were obtained from Dr. Katsuhisa Inoue (Nagoya City University). BioCoat™ 96-well poly-D-lysine 96-well plates were obtained from Corning Inc (Corning, NY). Fetal bovine serum was purchased from Sigma-Aldrich (St.Louis, MO). DMEM (Dulbecco's Modified Eagle Medium), Hygromycin B, Gentamicin and sodium pyruvate were obtained from Gibco life technologies (Waltham, MA). HBSS (Hank's Balanced Salt Solution), HEPES, 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid) were obtained from Lonza Inc. (Allendale, NJ). ^{14}C-Creatinine and ^{14}C-Metformin were purchased from Moravek Biochemical Inc. (Brea,CA). NP-40 Surfact-Amps™ Detergent Solution was purchased from Thermo Scientific (Rockford, IL). ^{3}H-cGMP, ^{3}H-para aminohippuric acid and ^{3}H-estrone sulfate were purchased from Perkin Elmer (Oat Brook, IL) and ^{3}H-penciclovir were purchased from American Radiolabeled Chemicals (St. Louis, MO).

LC/MS/MS Method

LC-MS/MS analyses for penciclovir were performed on a SCIEX Triple Quad 6500 mass spectrometer equipped with an IonDrive Turbo V ion source. The HPLC systems consisted of an Agilent 1290 Infinity binary pump. An Apricot/Sound Analytics ADDA autosampler was used for sample introduction. All instruments were controlled and synchronized by SCIEX Analyst

software (version 1.6.2 or higher) working in tandem with the ADDA software. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). Either a Phenomenex Kinetex C18 (2.6 μm, 2.1 × 30 mm) column with a C18 guard column was used or a Phenomenex Synergi Polar-RP (2.6 μm, 2.1 × 30 mm). The following gradient was used to elute samples using the C18 column: flow rate was set at 0.8 mL/min, 5% solvent B for 0.2 min, increasing to 95% B for 0.5 min, held at 95% B for 0.3 min, reduction to 5% B over 0.02 min, and held at 5% solvent B for 0.48 min (total run time 1.5 min). For the Polar-RP column, the gradient was: flow rate was set at 0.6 mL/min, 0% solvent B increasing to 70% B for 1 min, held at 70% B for 0.2 min, reduction to 0% B over 0.05 min, and held at 0% solvent B for 0.75 min (total run time 2.0 min). Quantitative analysis was performed in multiple reaction monitoring (MRM) mode. The MRM transition monitored for penciclovir was m/z 254.3/152.2 and the internal standard was 687.0/320.0. Results were analyzed using MultiQuant version 2.1 or 3.0.