Supplemental Data

Pharmacokinetics of Organic Cation Transporter 1 (OCT1) Substrates in Oct1/2 Knockout Mice and Species Difference in Hepatic OCT1-mediated Uptake

Bridget L. Morse, Anil Kolur, Loyd R. Hudson, Andrew T. Hogan, Lisa Hong Chen, Ryan M. Brackman, Geri A. Sawada, John K. Fallon, Philip C. Smith, Kathleen M. Hillgren

Eli Lilly and Company, Indianapolis, Indiana, USA (BLM, AK, LRH, ATH, LHC, RMB, GAS, KMH)

Division of Pharmacoengineering and Molecular Pharmaceutics, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA (JKF, PCS)

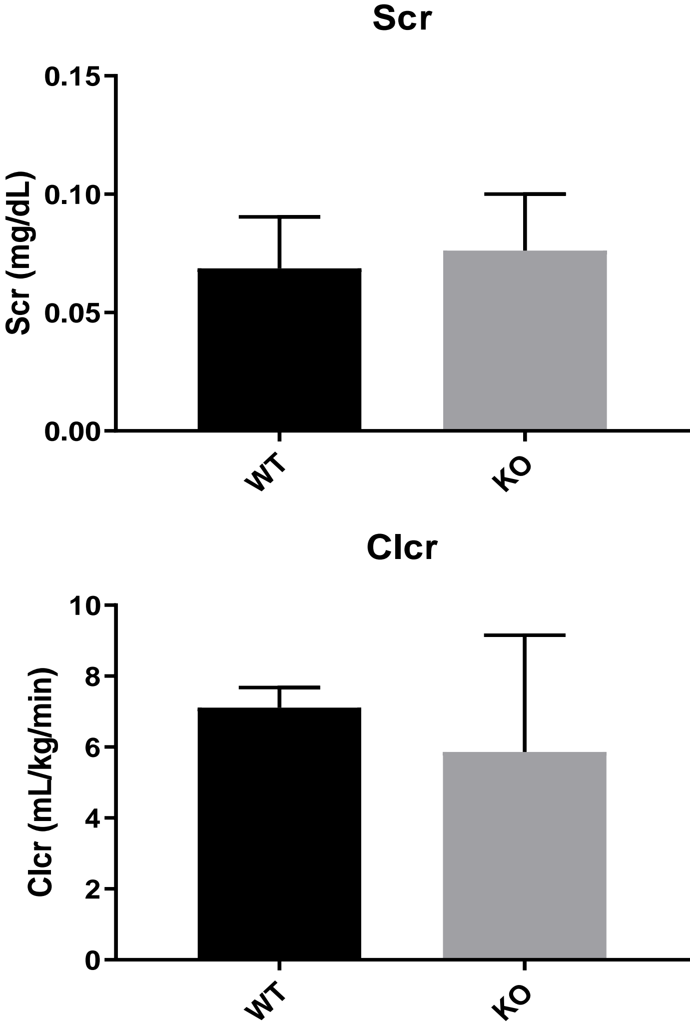
Drug Metabolism and Disposition

Supplemental Figure 1. Creatinine pharmacokinetics in wildtype and Oct1/2$^{-\backslash-}$ mice. SCr=serum creatinine. Clcr=creatinine clearance. WT=wildtype mice. KO=Oct1/2 knockout mice. Serum was taken from mice (n=4) at steady-state (0 and 8 hrs). Urine was collected from 0-8 hrs. Clcr was calculated as the amount in urine/area under the serum concentration-time curve calculated from 0-24 hours. Data presented as mean ± SD.

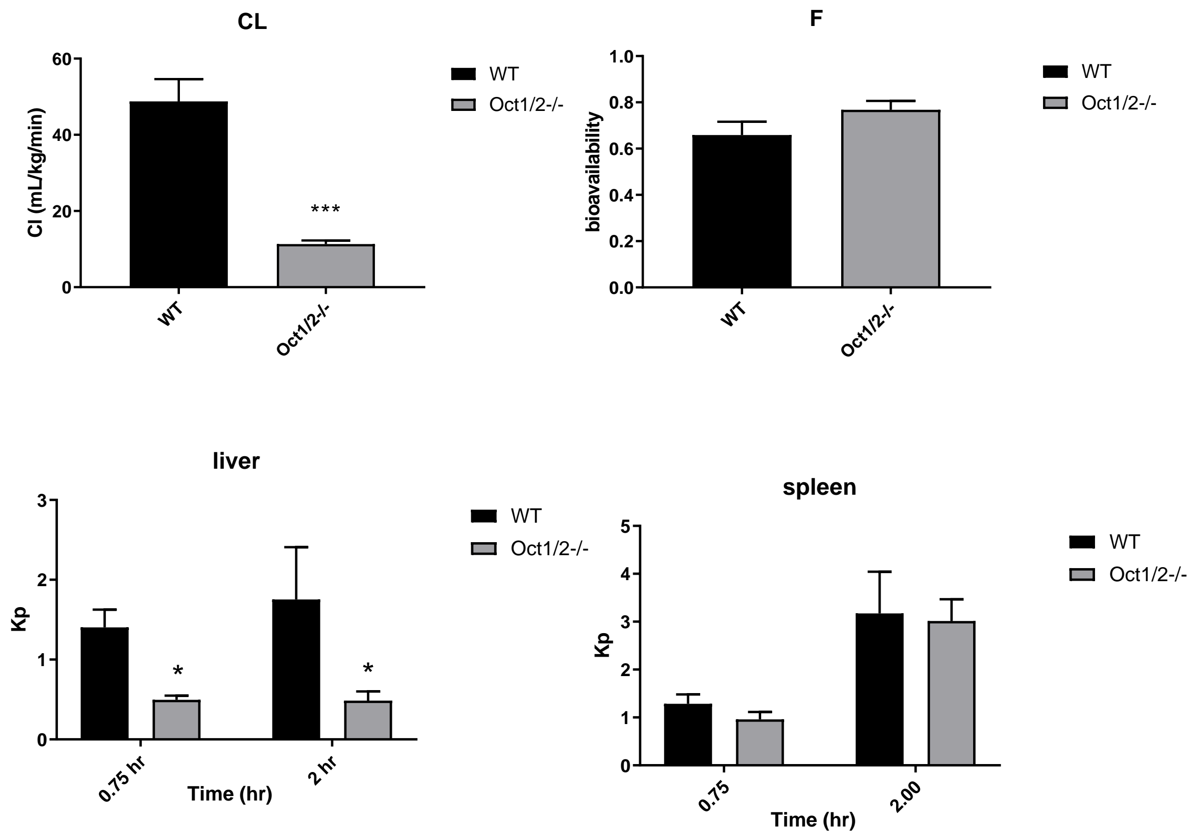
Supplemental Figure 2. Pharmacokinetics and tissue partitioning of metformin in wildtype and Oct1/2$^{-\backslash-}$ mice. Mice (n=4) were administered metformin intravenously (5 mg/kg) and orally 10 mg/kg) and blood samples collected. Separate mice were administered metformin intravenously and tissue and plasma collected at the indicated timepoints. Cl=total clearance. F=bioavailability. Kp=tissue partition coefficient. *p<0.05 using student's t-test, compared to WT. ***p<0.001 using student's t-test, compared to WT. Data presented as mean ± SD.

Supplemental Figure 3. A) Uptake kinetics of OCT1 substrates in OCT1- and OCT2-expressing cells. B) Inhibition of sumatriptan uptake by MPP+ in OCT1- and OCT2-expressing cells. Uptake data presented as mean ± SD.

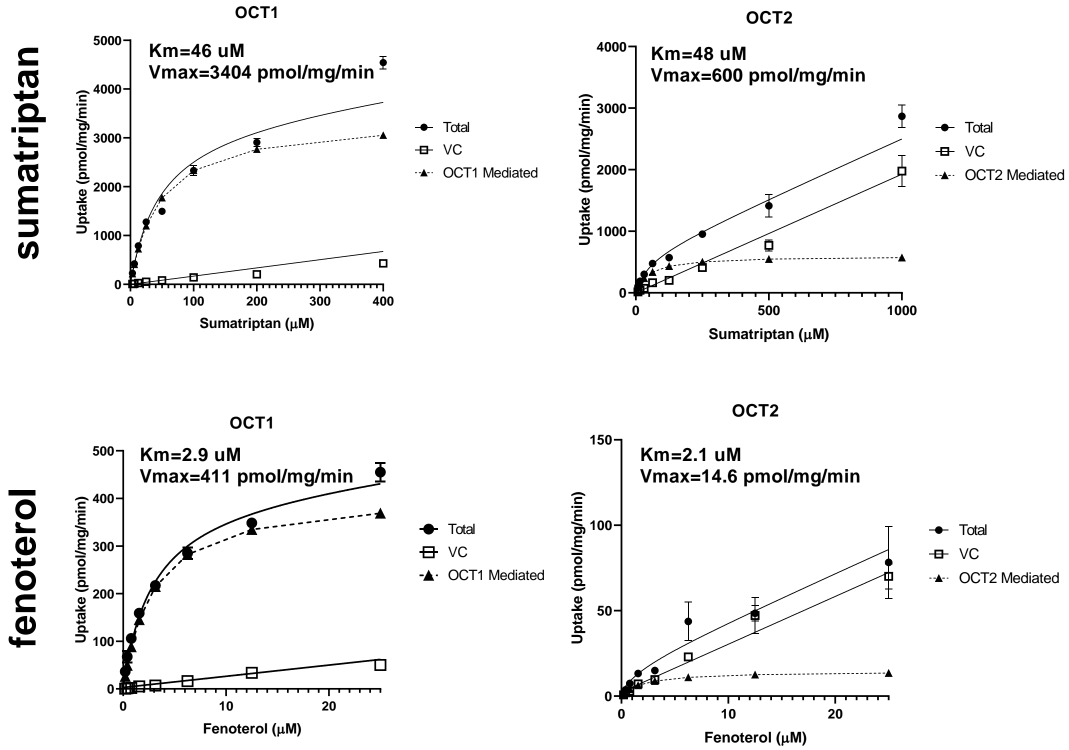
Supplemental Figure 1

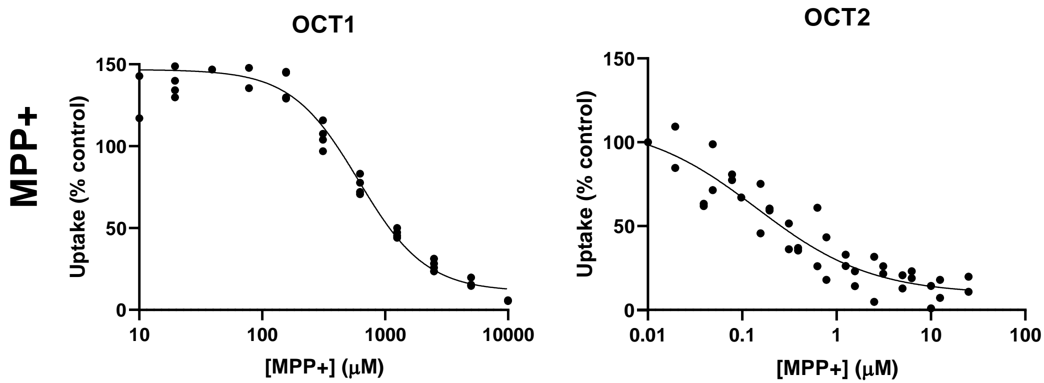


Supplemental Figure 2



Supplemental Figure 3





Supplemental Table 1. Peptides utilized for LC-MS/MS quantitation of individual mouse transporter proteins.

TRANSPORTER/MARKER	PEPTIDE
OCT1	ENTIYLQVQTGK
OCT2	WLISQNK
OCT3	GIALPETVEDVEK
OATP1A1/(3)/4/5	YLEQQYGK
OATP1A3/4/(6)	IYLGLPAALR
OATP1A5	EGLQDNVAR
OATP2A1	HLPGLLPSK
OATP3A1	SGELQGDEAQR
OATP12	GAVSNPAFGK
OATP1B2	SVQPELK
OATP2B1	LYVDIDR
OAT3	TALAVFGK
MDR1A	VVSYEEIVR
MDR1B	TVIAFGGQQK
P-GP	IATEAIENFR
BCRP	SSLLDVLAAR
MRP1	TPSGNLVNR
MRP2	LTIIPQDPILFSGNLR
MRP3	GALVAVVGPVGCGK
MRP4	LNTIIDSDK
BSEP	STALQLIQR
NTCP	GIYDGDLK
OST-ALPHA	NTLC[CAM]PIK
NAK ATPASE	VDNSSLTGESEPQTR

Supplemental Methods

LC-MS/MS details for measurement of each OCT1 substrate

Sumatriptan

Study samples were analyzed by LC-MS/MS using a Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS; Foster City, CA) equipped with a TurboIonSpray interface, and operated in positive ion mode. The analytes were chromatographically separated using a Betasil C18 20x2.1mm 5 micron Javelin HPLC column (Thermo Electron Corp.). The pumps were Shimadzu LC-10AD units with a SCL-10A controller (Kyoto, Japan), and a Gilson 215 liquid handler (Middleton, WI) was used as the autosampler. Water / trifluoroacetic acid / 1 M ammonium bicarbonate (2000:8:2, v/v) (Mobile Phase A), and acetonitrile/ trifluoroacetic acid / 1 M ammonium bicarbonate, (2000:8:2, v/v) (Mobile Phase B). The gradient profile from start to 0.2 min was 5%, 35% from 0.3 to 0.4 min, and 98% from 0.41 to 0.72 min. The flow rate was 1.5 mL/min. Chromatography was performed at ambient temperature, with flow directed to the mass spectrometer between 0.25 and 0.50 min. The selected reaction monitoring (SRM) (M+H) transition m/z 296.2 > 58.1 for Sumatriptan. Sumatriptan-d6 was used as stable label internal standard with SRM (M+H) transition m/z 302.2 > 64.2. The TurboIonSpray temperature was maintained at 740ºC, with collision, curtain, nebulizing, and desolvation gas (nitrogen) settings of 46, 10, 50, and 70, respectively. The ionspray voltage was set to 1500 V, while the respective declustering, entrance, and exit potentials were 80, 6, and 9.

Fenoterol

Study samples were analyzed by LC-MS/MS using a Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS; Foster City, CA) equipped with a TurboIonSpray interface, and operated in positive ion mode. The analytes were chromatographically separated using a Betasil C18 20x2.1mm 5 micron Javelin HPLC column (Thermo Electron Corp.). The

pumps were Shimadzu LC-10AD units with a SCL-10A controller (Kyoto, Japan), and a Gilson 215 liquid handler (Middleton, WI) was used as the autosampler. Water / trifluoroacetic acid / 1 M ammonium bicarbonate (2000:8:2, v/v) (Mobile Phase A), and acetonitrile/ trifluoroacetic acid / 1 M ammonium bicarbonate, (2000:8:2, v/v) (Mobile Phase B). The gradient profile from start to 0.2 min was 10%, 25% from 0.3 to 0.4 min, and 98% from 0.41 to 0.72 min. The flow rate was 1.5 mL/min. Chromatography was performed at ambient temperature, with flow directed to the mass spectrometer between 0.25 and 0.50 min. The selected reaction monitoring (SRM) (M+H) transition m/z 304.3 > 107.1 for Fenoterol. Fenoterol-d6 was used as stable label internal standard with SRM (M+H) transition m/z 310.5 > 141.1. The TurboIonSpray temperature was maintained at 740ºC, with collision, curtain, nebulizing, and desolvation gas (nitrogen) settings of 45, 10, 50, and 70, respectively. The ionspray voltage was set to 1500 V, while the respective declustering, entrance, and exit potentials were 80, 10, and 7.

Ondansetron

Study samples were analyzed by LC-MS/MS using a Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS; Foster City, CA) equipped with a TurboIonSpray interface, and operated in positive ion mode. The analytes were chromatographically separated using a Betasil C18 20x2.1mm 5 micron Javelin HPLC column (Thermo Electron Corp.). The pumps were Nexera LC-30AD units with a Shimadzu CMB-20A controller (Kyoto, Japan), and a PAL System (Zwingen, Switzerland) was used as the autosampler. Water / 1 M ammonium bicarbonate (2000:10, v/v) (Mobile Phase A), and methanol / 1 M ammonium bicarbonate, (2000:10, v/v) (Mobile Phase B). The gradient profile from start to 0.2 min was 42%, 72% from 0.3 to 0.4 min, and 98% from 0.41 to 0.72 min. The flow rate was 1.5 mL/min. Chromatography was performed at ambient temperature, with flow directed to the mass spectrometer between 0.25 and 0.50 min. The selected reaction monitoring (SRM) (M+H) transition m/z 294.2 >184.1 for Ondansetron. Ondansetron-d3 was used as stable label internal standard with SRM (M+H) transition m/z 297.2 > 187.0. The TurboIonSpray temperature was maintained at 740ºC, with

collision, curtain, nebulizing, and desolvation gas (nitrogen) settings of 35, 10, 50, and 70, respectively. The ionspray voltage was set to 1500 V, while the respective declustering, entrance, and exit potentials were 70, 10, and 13.

Tropisetron

Study samples were analyzed by LC-MS/MS using a Sciex API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS; Foster City, CA) equipped with a TurboIonSpray interface, and operated in positive ion mode. The analytes were chromatographically separated using a Betasil C18 20x2.1mm 5 micron Javelin HPLC column (Thermo Electron Corp.). The pumps were Nexera LC-30AD units with a Shimadzu CMB-20A controller (Kyoto, Japan), and a PAL System (Zwingen, Switzerland) was used as the autosampler. Water / trifluoroacetic acid / 1 M ammonium bicarbonate (2000:8:2, v/v) (Mobile Phase A), and acetonitrile/ trifluoroacetic acid / 1 M ammonium bicarbonate, (2000:8:2, v/v) (Mobile Phase B). The gradient profile from start to 0.2 min was 17%, 47% from 0.3 to 0.4 min, and 98% from 0.41 to 0.72 min. The flow rate was 1.5 mL/min. Chromatography was performed at ambient temperature, with flow directed to the mass spectrometer between 0.25 and 0.50 min. The selected reaction monitoring (SRM) (M+H) transition m/z 285.3 >124.3 for Tropisetron. Tropisetron-d5 was used as stable label internal standard with SRM (M+H) transition m/z 290.3 > 124.0. The TurboIonSpray temperature was maintained at 740ºC, with collision, curtain, nebulizing, and desolvation gas (nitrogen) settings of 30, 10, 50, and 70, respectively. The ionspray voltage was set to 3000 V, while the respective declustering, entrance, and exit potentials were 80, 10, and 8.