List of Supplemental Materials

Supplemental Methods

Quantification of furosemide

For both plasma and urine samples, a working standard solution was prepared with 0.6 ng/µL of furosemide in methanol, and an internal standard solution contained 0.25 ng/µL of probenecid in methanol. Plasma unknown samples and quality-control samples were prepared for analysis by combining 20 µL of internal standard with 50 µL plasma or urine and 150 µL 0.1% formic acid in methanol. Calibration samples were prepared by combining 50 µL plasma free from furosemide, 20 µL internal standard, and 0 to 100 µL working standard solution plus enough of the prepared 0.1% formic acid methanol solution to make the final methanol volume 150 µL. Samples were vortexed for 30 seconds and centrifuged at 4°C for 10 minutes at 14,000 RCF and the supernatant was transferred to a 96-well plate for analysis. Urine unknown samples and quality-control samples were prepared for analysis by combining 20 µL of internal standard with 50 µL urine and 500 µL of 100 mM potassium phosphate buffer, pH 4. Calibration samples, which were made with urine free from furosemide, also contained 0 to 100 µL of the working standard solution. Samples were vortexed for 10 seconds, and then 2 mL of ethyl acetate was added. The tubes were shaken horizontally for 10 minutes. The phases were allowed to separate, and the organic phase was transferred to a glass tube and dried at 40ºC under a stream of nitrogen gas. The dried samples were reconstituted with 100 µL of 1:1 0.1% formic acid to methanol and transferred to a 96-well plate. For both plasma and urine samples, 1 µL of the prepared sample was injected onto a Shimadzu Nexera UPLC (Shimadzu Corporation, www.shimadzu.com) coupled to an AB Sciex 6500 Q-Trap tandem mass spectrometer (Sciex, sciex.com). Mobile phase A was 0.1% formic acid in water and mobile phase B was methanol. The flow rate was 0.3 mL/min and an elution gradient started at 25% B for 0.5 minutes, increased linearly to 95% by 2.0 minutes, held at 95% until 4.0 minutes, decreased to 25% by 4.25 minutes, and equilibrated until 7.0 minutes before next injection. Chromatographic separation was achieved with an Agilent Eclipse C-18 50 mm x 2.1 mm x 1.8 µm particle-size column (Agilent). The mass spectrometer operated in electrospray

mode with negative polarity. The following transitions were monitored: 328.6>204.8 m/z (furosemide) and 283.8>239.8.0 m/z (probenecid). The declustering potential was -5 V. The collision energy was -28 V for furosemide and -18 V for probenecid. The ion spray voltage was -4500 V with the source set to 550ºC.

Quantification of penciclovir

For plasma samples, a working standard solution contained 1.0 ng/µL penciclovir in methanol, and for urine samples, a working standard solution contained 160 ng/µL penciclovir in methanol. An internal standard solution contained 1.0 ng/µL of d_4-penciclovir in methanol. Unknown samples and quality-control samples were prepared for analysis by combining 20 µL of the internal standard solution with 50 µL plasma or urine and 150 µL methanol. Calibration samples were prepared by combining 50 µL of plasma or urine free from penciclovir, 20 µL of the internal standard solution, and 0 to 100 µL of working standard solution plus methanol to make the final methanol volume 150 µL. Samples were vortexed for 30 seconds and centrifuged at 4°C for 10 minutes at 14,000 RCF. The supernatant was transferred to a 96-well plate and 2 µL were injected onto an Agilent 1290 Infinity high-pressure liquid chromatography coupled to an Agilent Technologies 6410 triple-quadrupole tandem mass spectrometer. Chromatographic separation was achieved with a Thermo Scientific Hypercarb 100 mm x 2.1 mm column with a 5 µm particle-size and 80Å pore size. Mobile phase A was 0.1% formic acid in water and mobile phase B was methanol. The flow rate was 0.4 mL/min, and an elution gradient started at 2.5% B for 4.0 minutes, increased linearly to 80% by 10.0 minutes, then decreased to 2.5% by 10.5 minutes. The mass spectrometer was operated in electrospray ionization mode with positive polarity. The following m/z transitions were monitored: 254.2>152.0 (penciclovir) and 258.2>152.0 (d_4-penciclovir). The drying gas temperature was set to 350°C at a flow rate of 10 L/minute. The nebulizer gas was nitrogen at 35 psig. The capillary voltage was 4000 V with the quadrupole temperature set to 100°C. Peak integration was performed using MassHunter Quantitation software (Agilent) and the response was measured by peak height. A second-order polynomial of analyte peak height normalized by internal standard peak height given nominal mass in the sample was fit to the data with weighting by 1/x.

Supplemental Table 1. Exclusion criteria of the PROCLAIM study

- Age ≤18
- Currently receiving maintenance hemodialysis or peritoneal dialysis
- Current or previous solid organ transplantation
- Known allergy to any of the study medications, iodine, acyclovir, or sulfa containing medications
- Current or regular use of any of the study medications (furosemide, famciclovir, tenofovir, oseltamivir)
- Current or regular use of probenecid, cimetidine, or digoxin
- Pregnancy or lactation
- Liver cirrhosis or liver failure
- Heart failure: New York Heart Association class III or greater
- Voiding problems or requirement for self-catheterization
- Nephrotic syndrome: urine albumin >3 grams per day
- Non-English speaking
- Inability to provide written informed consent
- ALT greater than 3 times the upper limit for the test
- Hemoglobin <9 mg/mL
- Platelet count <100K
- Serum potassium >5.5 or <3.5 mEq/mL

Supplemental Table 2. Kidney clearances and laboratory characteristics of secretory solutes.[a]

	Median kidney clearance (mL/min, IQR)	Pearson correlation with iGFR[b]	Spearman correlation with iGFR[b]	Protein binding in healthy (%)[c]	Protein binding in CKD (%)[c]	Plasma LLQ (ng/mL)	Urine LLQ (ng/mL)	Intra-assay CV plasma (%)	Inter-assay CV plasma (%)	Intra-assay CV urine (%)	Inter-assay CV urine (%)	Median diurnal CV plasma (%, IQR)[d]
Pyridoxic acid	783 (489, 983)	0.83	0.84	83 ± 4	87 ± 1	1.5	21	3.4	4.7	5.7	5.8	19.5 (14.1, 26.0)
Isovalerylglycine	493 (320, 882)	0.73	0.78	6 ± 13	4 ± 7	0.6	16	7.1	7.3	5.4	5.9	25.2 (16.0, 32.4)
Tiglylglycine	338 (234, 565)	0.70	0.77	33 ± 20	24 ± 15	0.6	15	7.0	14.7	6.0	5.5	27.4 (18.1, 34.6)
Kynurenic acid	318 (222, 442)	0.74	0.78	97 ± 1	96 ± 2	0.7	6.7	4.1	5.5	5.6	8.6	17.0 (11.5, 21.2)
Xanthosine	215 (154, 313)	0.69	0.73	11 ± 14	15 ± 13	0.3	2.4	11.1	14.5	9.8	10.1	22.6 (17.7, 30.8)
Cinnamoylglycine	168 (98, 230)	0.82	0.82	91 ± 12	95 ± 3	0.2	0.7	4.5	5.4	4.9	4.5	36.0 (26.4, 54.2)
Indoxyl sulfate	60 (40, 82)	0.81	0.82	97 ± 1	93 ± 2	9.1	330	4.3	6.0	6.1	9.4	19.2 (10.7, 26.1)
p-cresol sulfate	24 (16, 30)	0.82	0.86	97 ± 1	96 ± 2	29	49	3.9	5.2	5.6	5.2	16.3 (11.7, 24.2)

[a] CV: coefficient of variation; IQR: inter-quartile range; iGFR: iohexol measurement of GFR; LLQ: lower limit of quantification.

[b] Pearson/Spearman correlations between secretory solute clearances and iohexol measurement of GFR, both not standardized to 1.73 m^2 body surface area.

[c] Protein binding percentage (mean ± SD) in 14 healthy persons with estimated GFR ≥ 90 mL/min/1.73 m^2 and no albuminuria and in 14 non-dialyzed patients with advanced CKD from the Seattle Kidney Study with estimated GFR < 15 mL/min/1.73 m^2.

[d] Diurnal CV was calculated using plasma samples drawn at baseline, 1 hour, 5 hour, 8 hour, and 24 hour after baseline.

Supplemental Table 3. Plasma concentration of secretory solutes during the study visit.

	Median plasma concentration (IQR), ng/mL				P for trend[c]
	Baseline[a]	1 hour[b]	5 hour[b]	8 hour[b]	
Pyridoxic acid	6.9 (3.9, 12.5)	5.6 (3.5, 11.0)	4.7 (3.3, 10.1)	4.9 (3.2, 11.6)	0.662
Isovalerylglycine	5.0 (3.3, 7.9)	4.9 (3.1, 7.4)	6.5 (3.9, 11.3)	6.0 (4.0, 11.3)	0.332
Tiglylglycine	6.5 (4.8, 11.9)	5.7 (4.2, 9.9)	8.0 (5.6, 13.2)	8.2 (6.1, 13.4)	0.302
Kynurenic acid	9.7 (7.3, 13.8)	8.8 (6.9, 13.2)	10.0 (7.3, 15.6)	9.6 (7.0, 14.0)	0.456
Xanthosine	6.9 (5.1, 10.5)	4.3 (3.4, 7.4)	5.5 (4.6, 7.8)	5.9 (4.8, 7.7)	0.063
Cinnamoylglycine	16.9 (5.4, 29.7)	15.5 (4.9, 24.9)	10.2 (2.6, 24.6)	9.9 (3.5, 18.7)	0.452
Indoxyl sulfate	1456.1 (961.5, 1810.0)	1280.0 (863.9, 1912.3)	1074.0 (850.8, 1695.1)	1110.2 (862.1, 1892.5)	0.110
p-cresol sulfate	4669.5 (2864.0, 6890.8)	4789.1 (2807.0, 6974.1)	4163.6 (2593.6, 7333.7)	3821.0 (2421.5, 7177.1)	0.834

[a] Before drug administration

[b] After drug administration

[c] P-values of the association between plasma concentration of secretory solutes and time was calculated from linear mixed-effects models with both random intercepts and random slopes.

Supplemental Table 4. Root mean square error (RMSE) of kidney clearance of medications predicted by iGFR or secretory solutes clearances. [a]

	Furosemide			Penciclovir	
	RMSE between predicted and measured drug clearance, ml/min	Difference in RMSE comparing iGFR to secretory clearance, ml/min (95% CI) [b, c]		RMSE between predicted and measured drug clearance, ml/min	Difference in RMSE comparing iGFR to secretory clearance, ml/min (95% CI) [b, c]
iGFR	27.4	/		76.5	/
$eGFR_{creatinine}$	30.0	-2.6 (-7.7, 3.1)		91.0	-14.5 (-29.4, -2.3)
Secretion score	25.4	2.0 (-4.7, 9.2)		56.3	20.2 (3.4, 38.9)
Individual clearance					
Pyridoxic acid	25.9	1.5 (-4.3, 6.8)		67.0	9.5 (-10.1, 29.9)
Isovalerylglycine	33.9	-6.5 (-14.5, 2.0)		66.7	9.8 (-14.5, 34.9)
Tiglylglycine	35.7	-8.3 (-15.6, 0.3)		84.6	-8.1 (-29.0, 18.4)
Kynurenic acid	30.8	-3.4 (-10.1, 4.0)		69.9	6.6 (-15.3, 28.6)
Xanthosine	36.1	-8.7 (-15.7, -0.2)		97.5	-21.0 (-38.0, -1.3)
Cinnamoylglycine	29.5	-2.1 (-8.6, 4.5)		74.8	1.7 (-15.7, 19.2)
Indoxyl sulfate	27.3	0.1 (-4.4, 3.9)		81.9	-5.4 (-16.3, 5.1)
p-cresol sulfate	27.2	0.2 (-5.2, 5.7)		66.2	10.3 (-4.3, 23.7)

[a] iGFR: glomerular filtration rate measured by iohexol clearance; $eGFR_{creatinine}$: estimated GFR based on serum creatinine concentrations from CKD-EPI equation; RMSE: root mean square error.

[b] 95% confidence intervals derived using leave-on-out cross validation with bootstrap with 500 iterations. Positive values indicate greater agreement for secretory solute clearances and negative values indicate greater agreement for iGFR.

[c] None of the differences was significant after correction for multiple comparisons using the Hommel method.

Supplemental Table 5. 1 – P_{30} of kidney clearance of medications predicted by iGFR or secretory solutes clearances. [a]

	Furosemide			Penciclovir	
	Percentage of estimates ≥30% from measured value, % (1- P_{30})	Difference in 1-P_{30} comparing iGFR to secretory clearance, % (95% CI)[b, c]		Percentage of estimates ≥30% from measured value, % (1- P_{30})	Difference in 1-P_{30} comparing iGFR to secretory clearance, % (95% CI)[b, c]
iGFR	28.3	/		18.5	/
$eGFR_{creatinine}$	30.2	-1.9 (-15.1, 17.0)		31.5	-13.0 (-31.5, 0.0)
Secretion score	22.6	5.7 (-3.8, 22.6)		11.1	7.4 (-5.6, 16.7)
Individual clearance					
Pyridoxic acid	30.2	-1.9 (-13.2, 17.0)		25.9	-7.4 (-20.4, 7.4)
Isovalerylglycine	47.2	-18.9 (-26.4, 5.7)		31.5	-13.0 (-27.8, 3.7)
Tiglylglycine	49.1	-20.8 (-28.3, 1.9)		35.2	-16.7 (-31.5, 1.9)
Kynurenic acid	34.0	-5.7 (-17.0, 15.1)		33.3	-14.8 (-24.1, 7.4)
Xanthosine	50.0	-21.7 (-31.3, 1.9)		35.2	-16.7 (-33.3, -1.9)
Cinnamoylglycine	28.8	-0.5 (-13.0, 17.7)		35.8	-17.3 (-28.5, 5.1)
Indoxyl sulfate	36.5	-8.2 (-14.8, 16.7)		24.5	-6.0 (-22.2, 7.4)
p-cresol sulfate	32.7	-4.4 (-13.2, 16.4)		20.8	-2.3 (-14.9, 10.8)

[a] iGFR: glomerular filtration rate measured by iohexol clearance; $eGFR_{creatinine}$: estimated GFR based on serum creatinine concentrations from CKD-EPI equation; 1-P_{30}: percentage of predicted estimates that differed by more than 30% from the measured medication clearance.

[b] 95% confidence intervals derived using leave-on-out cross validation with bootstrap with 500 iterations. Positive values indicate greater agreement for secretory solute clearances and negative values indicate greater agreement for iGFR.

[c] None of the differences was significant after correction for multiple comparisons using the Hommel method.