Caetano-Pinto et al.: Amplifying the Impact of Kidney Microphysiological Systems: Predicting Renal Clearance Using Mechanistic Modelling Based on Reconstructed Drug Secretion

Supplementary Data

Table of Contents
1 Primary human renal proximal tubule epithelial cells (RPTEC)........................................................................... 1
2 Antibodies, specificity evaluation and localization ................................................................................................. 2
3 Gene reference list, statistics and housekeeping genes .............................................................................................. 3
    Gene expression statistical analysis ............................................................................................................................. 3
4 Apparent permeability and trans-epithelial flux calculations ...................................................................................... 4
5 Shear stress calculations in kidney-MPS ...................................................................................................................... 5
6 MPS-chip renal tubule cell count ..................................................................................................................................... 6
7 Metformin and cidofovir perfusion cytotoxicity ......................................................................................................... 6
8 Quantification of trans-epithelial drug transport in the micro-perfusion platform .................................................... 7
    The micro-perfusion model ............................................................................................................................................. 7
    Assumptions and modelling steps ............................................................................................................................. 8
    Software implementation ............................................................................................................................................ 9
9 In vitro to in vivo extrapolation (IVIVE) ..................................................................................................................... 9
10 The steady-state model ............................................................................................................................................... 11
References .................................................................................................................................................................... 12

1 Primary human renal proximal tubule epithelial cells (RPTEC)

Tab. S1: Supplier specifications of the RPTEC and kidney tissue used

| RPTEC | Supplier | Biopredic (https://www.biopredic.com/) |
|-------|----------|----------------------------------------|
| Batch | RPT101029|                                        |
| Donor gender | Female | |
| Donor age | 50 years | |
| Specimen collection | Renal outer cortex nephrectomy | |
| Cell isolation | Percoll density-gradient centrifugation | |
| Storage | Cell culture medium containing 10% DMSO | |
| Quality control (supplier) | TEER measurements (170 Ω/cm²) and rhodamine efflux | |

| Renal cortex samples | Sample | Supplier | Donor sex | Age | Procedure |
|----------------------|--------|----------|-----------|-----|-----------|
| #1                  | BioIVT | 79       | Female    | Nephrectomy |
| #2                  | BioIVT | 68       | Male      | Nephrectomy |
| #3                  | Sahlgrenska Hospital | 65     | Female    | Nephrectomy |

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Antibodies, specificity evaluation and localization

| Primary antibody | Reference       | Species | Dilutions | 2D-plastic | Kidney-MPS |
|------------------|-----------------|---------|-----------|------------|------------|
| ZO1              | 610966/BD       | mouse   | 1:500     | 1:200      |            |
| OCT2             | HPA008567/Sigma | rabbit  | 1:500     | 1:200      |            |
| P-gp             | ab170904/Abcam  | rabbit  | 1:500     | 1:200      |            |
| Na⁺K⁺-ATPase     | ab196884/Abcam  | rabbit  | 1:100     | 1:100      |            |
| Acetylated tubulin | T12-11/Sigma | mouse   | 1:500     | 1:200      |            |
| OAT1             | AB131087/Abcam  | rabbit  | 1:500     | 1:100      |            |

**Secondary antibody**

| Secondary antibody | Reference | Species | Dilutions | 2D-plastic | Kidney-MPS |
|--------------------|-----------|---------|-----------|------------|------------|
| Alexa Fluor 555 anti-mouse | A21424 | goat    | 1:500     | 1:100      |            |
| Alexa Fluor 555 anti-rabbit | A21429 | goat    | 1:500     | 1:100      |            |

Secondary antibodies were co-incubated with Phalloidin-488 (anti-F-actin; 1:200) and Hoechst 33342 (50 mM; 1:1000).

**Fig. S1: Specificity of drug transporter antibodies evaluated in overexpression cell lines**

Commercially available antibodies against membrane drug transporters are often reported as lacking specificity for their intended targets. To confirm that the antibodies used in this study recognize the drug transporters analyzed, immunostainings were performed in cell lines overexpressing OCT2 (HEK), OAT1 (HEK) or P-gp (MDCK). HEK-OCT2 (A) and HEK-mock transfected cells (D) were used to test the OCT2 antibody (dil. 1:500); HEK-OAT1 FlpIn (B) and HEK-FlpIn mock transfected cells (Zou et al., 2018) (E) were used to test the OAT1 antibody (dil. 1:500). MDCK-P-gp (C) and MDCK wild type cells (F) were used to test the P-gp antibody (dil. 1:1000). Secondary antibodies were used diluted 1:500 in combination with Hoechst 33342 1:1000.
Fig. S2: Cellular localization of OCT2 and P-gp in RPTEC in 2D-plastic culture

The spatial localization of OCT2 and P-gp was determined using reconstructed confocal Z-stacks consisting of eight frames each (2 μm apart) after staining for f-actin (Phalloidin 488: green) and OCT2 or P-gp (Alexa555: red). F-actin is seen delineating the cell boundaries in the orthogonal views. OCT2 is mostly localized intracellularly and does not colocalize with the membrane (A). P-gp is expressed both intracellularly and in the membrane, evident from the yellow stain that results from f-actin and P-gp co-localization (B).

3 Gene reference list, statistics and housekeeping genes

Tab. S3: List of renal proximal tubule genes analyzed

| Gene symbol [Protein name] | Hs-code (Thermo Fisher) | Gene name                                      |
|---------------------------|-------------------------|------------------------------------------------|
| AQP1                      | Hs01028916_m1           | aquaporin 1 (Colton blood group)               |
| CUBN                      | Hs00153607_m1           | cubilin                                        |
| HNF4A                     | Hs00153607_m1           | hepatocyte nuclear factor 4 alpha              |
| GGT1                      | Hs00980756_m1           | gamma-glutamyltransferase 1                    |
| HPRT1                     | Hs02800695_m1           | hypoxanthine phosphoribosyltransferase 1       |
| LRP2 [Megalin]            | Hs00189742_m1           | LDL receptor related protein 2                 |
| ABCB1 [P-gp]              | Hs00184500_m1           | ATP binding cassette subfamily C member 4      |
| SLC22A2 [OCT2]            | Hs01010726_m1           | solute carrier family 22 member 2              |
| SLC22A6 [OAT1]            | Hs00537914_m1           | solute carrier family 22 member 6              |
| SLC22A8 [OAT3]            | Hs00188599_m1           | solute carrier family 22 member 8              |
| SLC47A1 [MATE1]           | Hs00217320_m1           | solute carrier family 47 member 1              |
| SLC47A2 [MATE2-K]         | Hs00945652_m1           | solute carrier family 47 member 2              |
| SLCO4C1 [OATP4C1]         | Hs00698884_m1           | solute carrier organic anion transporter family member 4C1 |
| SLC5A1 [SGLT1]            | Hs01573793_m1           | solute carrier family 5 member 1               |
| SLC5A2 [SGLT2]            | Hs00894642_m1           | solute carrier family 5 member 2               |
| GAPDH                     | Hs00427620_m1           | glyceraldehyde-3-phosphate dehydrogenase       |

Gene expression statistical analysis

Statistically significant differences between -ΔCt values were determined for each sample relative to 2D-plastic using unpaired t-tests corrected for multiple comparisons using the Holm-Sidak method, assuming the same scatter (SD) among samples, with α = 0.05. Calculations were performed using GraphPad Prism 8.
Tab. S4: Gene expression statistical analysis
Different samples (n = 3) were analyzed using unpaired t-tests relative to 2D-plastic using -ΔCt values. The table summarizes the adjusted P-value and statistical significance differences (P < 0.05) (Signf.)

| Gene   | MPS-day 2 | MPS-day 7 | 2D-transwell |
|--------|-----------|-----------|--------------|
|        | P value   | Signf.    | P value      | Signf.    | P value | Signf. |
| ABCC4  | 0.9723    | No        | 0.9431       | No        | 0.7031  | No     |
| AQP1   | 0.7684    | No        | 0.1161       | No        | 0.7377  | No     |
| CUBN   | 0.4514    | No        | 0.1255       | No        | 0.5749  | No     |
| GAPDH  | 0.8481    | No        | 0.8824       | No        | 0.9384  | No     |
| GGT1   | 0.2819    | No        | 0.0469       | No        | 0.4048  | No     |
| LRP2   | 0.6622    | No        | 0.0350       | No        | 0.4052  | No     |
| SLC47A1| 0.0001    | Yes       | < 0.0001     | Yes       | 0.0755  | No     |
| ABCB1  | 0.0212    | Yes       | 0.0709       | No        | 0.2360  | No     |
| SLC12A3| 0.6481    | No        | 0.6679       | No        | 0.9894  | No     |
| SLC22A2| 0.4093    | No        | 0.6841       | No        | 0.4431  | No     |
| SLC47A2| 0.0126    | Yes       | 0.0002       | Yes       | 0.8477  | No     |
| SLC5A2 | 0.7438    | No        | 0.7349       | No        | 0.9144  | No     |
| SLC04C1| 0.5744    | No        | 0.5095       | No        | 0.8774  | No     |
| HNF4A  | 0.0235    | Yes       | 0.0065       | Yes       | 0.0064  | Yes    |

Tab. S5: Absolute gene expression (Ct values) of the different samples analyzed (n = 3)

| Gene   | 2D-plastic | 2D-transwell | MPS-day 2 | MPS-day 7 | Kidney cortex |
|--------|------------|--------------|-----------|-----------|---------------|
|        | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| ABCC4  | 23.24 | 0.27 | 23.78 | 0.08 | 24.25 | 0.26 | 23.71 | 0.82 | 26.80 | 1.48 |
| AQP1   | 23.74 | 0.27 | 23.43 | 0.73 | 24.45 | 0.41 | 22.37 | 0.91 | 22.98 | 1.64 |
| CUBN   | 30.51 | 0.41 | 31.26 | 0.58 | 30.70 | 0.23 | 29.18 | 0.15 | 24.07 | 1.38 |
| GGT1   | 22.80 | 0.32 | 21.90 | 0.49 | 22.62 | 0.57 | 20.90 | 0.23 | 22.97 | 1.67 |
| LRP2   | 31.11 | 2.75 | 32.19 | 0.68 | 31.67 | 1.20 | 29.06 | 1.17 | 22.59 | 1.35 |
| SLC47A1| 33.30 | 0.20 | 31.26 | 0.38 | 29.66 | 1.15 | 27.17 | 1.13 | 23.42 | 1.23 |
| ABCB1  | 21.56 | 0.22 | 23.06 | 0.18 | 25.31 | 0.11 | 24.34 | 0.60 | 25.19 | 1.19 |
| SLC12A3| 21.41 | 0.55 | 21.48 | 0.49 | 21.94 | 0.48 | 21.44 | 0.76 | 22.10 | 0.80 |
| SLC22A2| 25.37 | 0.67 | 26.37 | 0.37 | 27.37 | 0.78 | 26.42 | 0.47 | 25.22 | 1.56 |
| SLC47A2| 30.14 | 1.13 | 30.46 | 0.82 | 28.26 | 1.06 | 25.93 | 0.75 | 23.66 | 0.92 |
| SLC5A2 | 34.79 | 0.19 | 28.79 | 5.08 | 33.04 | 2.97 | 31.12 | 2.77 | 24.84 | 2.36 |
| SLC04C1| 22.16 | 0.53 | 22.06 | 0.85 | 22.57 | 0.25 | 21.91 | 0.86 | 25.71 | 1.28 |
| SLC22A6| -     | -    | -     | -    | 34.17 | 0.64 | 29.26 | 0.85 | 22.23 | 1.03 |
| SLC22A8| -     | -    | -     | -    | -     | -    | 24.85 | 1.58 |           |
| HNF4A  | 31.99 | 1.03 | 28.27 | 0.07 | 28.85 | 1.30 | 27.25 | 1.01 | 22.05 | 0.93 |
| HPRT1  | 23.97 | 1.18 | 24.06 | 0.72 | 25.03 | 0.39 | 24.53 | 0.68 | 26.13 | 0.94 |
| GAPDH  | 16.77 | 1.09 | 16.95 | 0.26 | 18.04 | 0.66 | 17.15 | 0.30 | 21.46 | 1.23 |
| TBP    | 22.27 | 1.68 | 25.71 | 0.93 | 25.28 | 0.25 | 24.94 | 0.79 | 27.09 | 0.50 |

4 Apparent permeability and trans-epithelial flux calculations

The apparent permeability ($P_{\text{app}}$) of metformin and cidofovir was determined in a conventional 2D-transwell assay, in the apical-to-basolateral (A2B) direction and the basolateral-to-apical (B2A) direction. The efflux ratio (ER) was determined as $P_{\text{app(B2A)}}/P_{\text{app(A2B)}}$. An ER > 2 indicates that active transport is involved in the drug permeability.

$$ P_{\text{app}} = \frac{d\Delta C}{dt.A.C_i} $$

$P_{\text{app}}$ is defined as the change in concentration ($d\Delta C$) in the recipient compartment (A or B) over time ($dt$), crossing a barrier area (cell grow surface: $A$) relative to the initial concentration ($C_i$) in the donor compartment (A or B). Units: cm.s$^{-1}$.10$^{-6}$.
Tab. S6: Metformin and cidofovir $P_{app}$ values (cm.s$^{-1}$·10$^{-6}$) obtained from 2D-transwell experiments

Values represent three independent experiments performed in triplicate and are expressed as mean ± standard deviation. Efflux ratio (ER) is defined as $B_2A / A_2B$.

| Metformin          | Inhibited (Metformin + Imipramine) | Cidofovir                  | Inhibited (Cidofovir + Probenecid) |
|--------------------|-----------------------------------|-----------------------------|-------------------------------------|
|                    |                                   | A2B                         | A2B                                 |
| A2B                | 14.4 ± 13.3                       | 14.0 ± 6.6                  | 12.3 ± 16.3                         |
| B2A                | 13.7 ± 16.7                       | 13.7 ± 8.4                  | 9.0 ± 11.3                          |
| ER                 | 0.95                              | ER                          | 0.73                                |

**Fig. S3**: RPTEC paracellular permeability in the presence of metformin or cidofovir in the presence and absence of inhibitors

Fluorescent tracer lucifer yellow (LY) was used to determine RPTEC paracellular monolayer permeability when cultured in 2D-transwells. The monolayers were considered tight when LY leakage ≤1%. Both in presence of metformin or cidofovir with or without inhibitors, LY leakage is maintained at around 1% after 1 h incubation. At 2 h incubation, LY leakage increases across all treatments, indicating that the RPTEC monolayer loses some integrity. To ensure a tight RPTEC monolayer, metformin and cidofovir permeability assays were performed at 1 h incubation.

Trans-epithelial flux ($J$) calculations were performed to evaluate the movement of drugs from B2A in 2D-transwell and kidney-MPS. This permeability analysis accounts for the quantity of a drug ($m$) crossing the area of a barrier ($A$) to a recipient compartment over time ($t$), independent of the initial concentration in the donor compartment. Units: µmol.cm$^{-2}$.min$^{-1}$.

$$J = \frac{m}{A \cdot t}$$

**5 Shear stress calculations in kidney-MPS**

To estimate the shear stress experienced by RPTEC in the kidney-MPS chip, an online microfluidic flow rate and shear stress calculator was used (https://darwin-microfluidics.com/blogs/tools/microfluidic-flowrate-and-shear-stress-calculator). The dimensions of the tubule embedded in the MPS chip matrix were used (diameter: 125 µm; length: 5.8 mm), together with a viscosity of 0.7978 cP. Flow rates of either 0.5 µL/min (kidney-MPS culture conditions) or 1 µL/min (kidney-MPS assay conditions) were used to estimate shear stress.
Tab. S7: Shear stress calculation and laminar flow parameters

| Parameter          | 0.5              | 1                |
|--------------------|------------------|------------------|
| Flow rate          | 0.5 µL/min       | 1 µL/min         |
| Pressure           | 0.064 mbar       | 0.129 mbar       |
| Resistance         | 1.287 mbar.min/µL| 1.287 mbar.min/µL|
| Reynold number     | 0.106            | 0.213            |
| Wall shear stress  | 0.347 dyne/cm²   | 0.693 dyne/cm²   |
| Velocity           | 0.679 mm/s       | 1.358 mm/s       |

6 MPS-chip renal tubule cell count

Fig. S4: Depiction of the cell count workflow in kidney-MPS using ImageJ

Cell density in the renal tubule was estimated by counting the number of nuclei present in renal tubules. The renal tubule was split into 9 sections (A), and confocal stacks of 25 images (20x) from the midsection to the boundary of the tubule were acquired using a CV7000 imager (B-C). In ImageJ image stacks were projected to compile all structures present in one section (B), and the nucleus number was calculated. ImageJ instructions used were as follows:

1. Load image. Image → Type → 8 bit colour
2. Process → Binary → make binary
3. Process → Binary → Watershed
4. Analyse → analyse particles
5. Show: outlines
6. Display results
7. Summarize
8. In situ show.

The number of nuclei estimated reflects ½ of a tubule, and assuming a homogenous distribution of cells in the tubules, the values were doubled to estimate the whole renal tubule. Confocal stacks of the whole circumference of the renal tubule were not used since this would skew the total number of cells given that both the nucleus from the bottom and top half of the tubule are super-imposed.

7 Metformin and cidofovir perfusion cytotoxicity

To evaluate any cytotoxic effects in the kidney-MPS after perfusion with metformin or cidofovir, live-dead assays were applied. Assays were performed after a 6-h perfusion with metformin or cidofovir together with the inhibitors imipramine or probenecid, respectively.
Fig. S5: Kidney-MPS live-dead assays
Chips were perfused via the renal tubule with propidium iodide (PI, 1 µg/mL, panels 1), calcein-AM (1 µM, panels 2) and Hoechst 33342 for 1 h (1:1000, panels 3). Non-viable cells with their membrane integrity compromised incorporate PI (red), viable cells metabolize calcein-AM and retain calcein (green), nuclei are highlighted in blue. Cytotoxicity was not observed in the renal tubules perfused with metformin (A1-3) or cidofovir (B1-3), evident from the lack of PI accumulation. In a renal tubule with compromised viability (C1-3), PI permeates into cells, calcein metabolism is limited and nuclear stain is reduced due to cell loss.

8 Quantification of trans-epithelial drug transport in the micro-perfusion platform

The micro-perfusion model
A semi-mechanistic modelling approach, schematically illustrated in Figure 6A (main text), was developed to evaluate drug disposition in the kidney-MPS chip. The model considers the net diffusion of drug cross the physical extracellular matrix volume section \( V_{tot} \) separating the loading and renal microfluidic channels, and further the basolateral-to-apical transport into the renal channel (Tab. S8). \( V_{tot} \) is split into \( n_r \) well-stirred transit compartments of equal volume \( V_{tr} = V_{tot}/n_r \) to accommodate for the transit time through the matrix. The flux of drug at any time \( t \) into the first transit compartment is proportional to the concentration \( C_l(t) \) in the loading channel, as defined by a first-order rate constant \( Q_{tr}[\mu\text{L/min}] \) (Eq. S1). The same rate constant further defines flux into subsequent transit volumes and, in the cell-free chip presenting no epithelial cell barrier to passage, also transport into the renal channel (Eq. S2-S4). Drug is in turn leaving the renal channel at a rate governed by the chip perfusion rate \( Q_p[\mu\text{L/min}] \) on its path to the outlet collection port. In the presence of a RPTEC monolayer, drug translocates from the extracellular matrix to the renal channel either by diffusion down its concentration gradient (trans- or paracellularly) or by transporter-mediated secretion, categorized here as ‘passive’ and ‘active’ transport, respectively (Eq. S5). We expect the amount of drug transported to depend on the tubular area formed by the RPTECs, and hence define the rate constants as permeability-surface area products, denoted \( PS_p \) and \( PS_a[\mu\text{L/min}] \) for active and passive transport, respectively. A set of ordinary differential equations following mass-action principles forms a mathematical representation of the above. The rate by which the amount of drug \( X_{tr,i} \) changes over time is given by

\[
\frac{dX_{tr,1}}{dt} = Q_{tr} \times (C_l(t) - \frac{X_{tr,1}}{V_{tr}})
\]

Eq. S1
for the first transit compartment \( i = 1 \), where \( C_l(t) \) represents the concentration in the loading channel. Change in subsequent compartments \( i = 2:ntr-1 \) follows

\[
\frac{dX_{tr,i}}{dt} = \frac{Q_{tr}}{V_{tr}} \times (X_{tr,i-1} - X_{tr,i}),
\]

Eq. S2

whereas

\[
\frac{dX_{tr,n}}{dt} = \frac{1}{V_{tr}} \times (Q_{tr} \times X_{tr,n-1} - \max(Q_{tr}PS_a + PS_p) \times X_{tr,n})
\]

Eq. S3

defines flux into and out of the last transit compartment \( i = ntr \). The rate equation follows the assumption that sink conditions apply, with continuous flow through the tubule, and passive reabsorption is negligible. Following the reasoning above, change to the amount in the renal channel \( X_r \) is expressed as

\[
\frac{dX_r}{dt} = Q_{tr} \times \frac{X_{tr,n}}{V_{tr}} - Q_p \times \frac{X_r}{V_{ch}}
\]

Eq. S4

at empty cell-free chip conditions, whereas for the situation with a renal tubule, \( X_r \) is governed by

\[
\frac{dX_r}{dt} = (PS_a + PS_p) \times \frac{X_{tr,n}}{V_{tr}} - Q_p \times \frac{X_r}{V_{ch}}
\]

Eq. S5

where \( Q_p, PS_p \) and \( PS_a \) represent the perfusion rate, and active and passive permeability-surface areas, respectively.

Assumptions and modelling steps
In the micro-perfusion model described, kinetic parameters \( Q_{tr}, PS_p \) and \( PS_a \) and number of transit compartments \( ntr \) are not directly given by experimental conditions (Tab. S8) and need estimation through optimization of a likelihood function of the fit to observed concentrations. Identification required the following general assumptions: i) flux of drug through the loading channel and subsequent dispersion into the extracellular matrix in the presence of a RPTEC monolayer can be approximated in a cell-free chip setup, ii) concentration-time profiles at the outlet ports of each circuit are useful surrogates for profiles in the channels through the matrix chamber, and iii) the loading and renal channels can be considered well-mixed compartments.

Tab. S8: Model parameters defining experimental conditions and chip geometries

| Parameter                      | Symbol | Unit  | Value |
|-------------------------------|--------|-------|-------|
| Perfusion rate                | \( Q_p \) | \( \muL/min \) | 1.0   |
| Channel diameter              | \( d_{ch} \) | \( mm \) | 0.125 |
| Channel length                | \( l_{ch} \) | \( mm \) | 5.8   |
| Channel volume                | \( V_{ch} \) | \( \muL \) | 0.0712|
| Channel separation            |        | \( mm \) | 1.0   |
| ECM volume separating the microfluidic channels\* | \( V_{tot} \) | \( \muL \) | 0.725 |

* Product of channel length, diameter, and distance separating the channels.

The loading outlet profile after perfusion of drug through a cell-free chip over 6 hours demonstrates significant overlap to observations from chip with RPTEC tubule ± selective inhibitors (Fig. S6). This suggests that of the continuously introduced drug, the fraction dispersed into the extracellular matrix is relatively insensitive to the experimental condition over this time period, supporting assumption i). For reasons indicated above, including the consequence of flow profile and interactions with material, we expect a broadening of the concentration-time build-up along the length of each microfluidic circuit, also downstream of the matrix chamber. However, as loading and renal profiles are expected to be similarly convoluted, actual channel profiles – provided linear kinetics – will be interchangeable with observed port profiles in assessing exchange across the channels, in support of assumption ii). Finally, given the small volume and short residence time in the matrix loading and renal channels (< 0.1 min at experimental conditions), influence of axial concentration gradients is negligible, in line with assumption iii).
Parameters defining renal secretion in the MPS system, $PS_p$ and $PS_a$, ultimately used to predict human renal clearance, were estimated for each drug by a sequential fitting procedure. The cell-free chip profiles were used to calibrate the model’s baseline behavior. Firstly, the loading channel $C_l(t)$ was modelled empirically by curve-fitting of a sigmoidal three-parameter function to the loading channel outlet concentration.

$$C_l(t) = \frac{Top}{1 + 10^{(\log(t_{50}) - \log(t))\gamma}} \quad \text{Eq. S6}$$

Definition of $Top$ (concentration at the plateau), $t_{50}$ (time at which $C_l = Top/2$) and $\gamma$ (slope factor) allowed for simulation of the concentration driving flux into the extracellular matrix (Eq. S1) at any time $t$. This in turn enabled subsequent estimation of $Q_r$ by fitting the micro-perfusion model to the corresponding renal outlet profile (Fig. 6B1,C1, main text). Keeping $C_l(t)$ and $Q_r$ frozen, basolateral-to-apical transport of the epithelial cell layer, represented by parameters $PS_p$ and $PS_a$, was assessed by simultaneous fit to renal channel outlets in the absence and presence of selective inhibitors of the carrier-mediated pathway (Fig. 6B2,C2, main text). Parameter estimates for metformin and cidofovir are collected in Table 1 (main text). Estimates were found to be insensitive to number of transit compartments ≥ 3. Reported estimates were obtained for $n_r = 3$.

**Software implementation**

The micro-perfusion model was implemented in MATLAB (Release 2017a, The MathWorks, Inc., Natick, MA, US). Fitting applied a naïve pooled approach using the non-linear least squares solver `lsqnonlin` with the default trust-region-reflective algorithm. Model selection was guided by a composite of likelihood function optimization and visual inspection of the residual graphs. Confidence intervals were generated by Monte-Carlo simulations ($n = 500$) of concentration-time profiles on basis of parameter values randomly chosen from the multivariate normal distribution of the estimates, generated from the `mvnrnd` function, as implemented in MATLAB. At each simulated timepoint, upper and lower bounds were given by the 97.5th and 2.5th percentiles, respectively.

**9 In vitro to in vivo extrapolation (IVIVE)**

The renal clearance in human was predicted from $PS_p$ and $PS_a$ on the basis of the tubular surface area in the microfluidic system relative to that of the in vivo physiology following the scaling method established by Kunze et al. (2014). In brief, the approach expresses kidney organ clearance $CL_{r,org}$ as the net result of glomerular filtration $CL_{r,fil}$, tubular secretion $CL_{r,sec}$, and fractional tubular reabsorption $f_{reab}$.

$$CL_{r,org} = (CL_{r,fil} + CL_{r,sec}) \times (1 - f_{reab}) \quad \text{Eq. S9}$$

Filtration is calculated from the glomerular filtration rate $GFR$ and the fraction unbound in blood $fu_b$

$$CL_{r,fil} = fu_b \times GFR, \quad \text{Eq. S10}$$
whereas secretion is derived from the renal blood flow rate $Q_{r,b}$, $f_{ub}$ and the scaled intrinsic clearance of tubular transport $CL_{int,sec}$

$$CL_{sec} = \frac{Q_{r,b} \times f_{ub} \times CL_{int,sec}}{Q_{r,b}+f_{ub} \times CL_{int,sec}}$$

Eq. S11

applying the well-stirred liver model concept. Reabsorption from the tubule fluid is calculated as

$$f_{reab} = \frac{CL_{int,sec}}{GFR+CL_{int,sec}}$$

Eq. S12

where the intrinsic clearance of reabsorption in vivo $CL_{int,sec}$ constitutes the passive portion of $CL_{int,sec}$. The in vivo intrinsic clearances were upscaled from the MPS determined permeability-surfaces areas

$$CL_{int,sec} = (PS_a + PS_p) \times \frac{SA_{vivo}}{SA_{MPS}}$$

Eq. S13

$$CL_{int,reab} = PS_p \times \frac{SA_{vivo}}{SA_{MPS}}$$

Eq. S14

where $SA_{vivo}$ is the estimated total surface area of a human proximal tubules and $SA_{MPS}$ is the surface area of the RPTEC tubule layer in the microphysiological system:

$$SA_{vivo} = \frac{\pi \times d_{PT} \times l_{PT} \times n_{neph} \times n_{kid}}{BW}$$

Eq. S15

$$SA_{MPS} = \pi \times d_{ch} \times l_{ch}$$

Eq. S16

Physiological values (Tab. S9) for the proximal tubule diameter $d_{PT}$ and length $l_{PT}$, number of nephrons per kidney $n_{neph}$, number of kidneys $n_{kid}$, and body weight $BW$ were taken from literature (Lote, 2013) following the steps of Kunze et al. (2014). Sensitivity of metformin and cidofovir predicted renal clearance to variation of in vitro (MPS surface area) and physiological (in vivo surface area, GFR and renal blood flow) scaling parameters are shown in Figure S7. A 3-fold change of each parameter resulted in predicted renal clearance within a factor of 2 of the point estimate, with the expectation of GFR for which the clearance was within a factor of 3.

Tab. S9: Physiological numbers for estimation of a human proximal tubule surface area $SA_{vivo}$ (adapted from Kunze et al., 2014)

| Parameter                  | Symbol | Unit     | Value    |
|----------------------------|--------|----------|----------|
| PT length                  | $l_{PT}$ | mm       | 15.0     |
| PT diameter                | $d_{PT}$ | mm       | 0.070    |
| Number of nephrons/kidneys | $n_{neph}$ | --       | 1.50E+06 |
| Number of kidneys          | $n_{kid}$ | --       | 2        |
| Glomerular filtration rate | GFR    | mL/min   | 120      |
| Renal blood flow           | $Q_{r,b}$ | mL/min   | 1.20E+03 |
Fig. S7: Sensitivity analysis
Sensitivity of metformin (top) and cidofovir (bottom) predicted renal clearance to variation of individual in vitro (MPS surface area) and physiological (in vivo surface area, GFR and renal blood flow) scaling parameters. Best-fit simulations and 95% confidence range indicated by solid lines and shaded areas, respectively, as each parameter is varied. Red line indicates the point estimate for the renal clearance.

10 The steady-state model
The permeability-surface area products ($P_{\text{passive}}$ and $P_{\text{active}}$) were derived at steady-state, when drug concentration in the loading channel reaches a constant input, approximately 200 minutes after perfusion is initiated. In Equation S7, $Cd$ and $Cr$ are approximated by the loading and renal channel outlet concentrations, and $Vr/t$ is the flow rate (1 $\mu$L/min in this setup). The apparent permeability coefficient ($P_{\text{app}}$) is defined in Equation S8, where $SA$ corresponds to the surface area.
\[ PS = \frac{V_r \times Cr_{ss}}{t \times Cd_{ss}} \quad \text{Eq. S7} \]

\[ P_{app} = \frac{V_r \times Cr}{t \times SA \times Cd} \quad \text{Eq. S8} \]

Tab. S10: Permeability parameters, derived using the steady-state model
The receiver volume \((V_r)\) considered for the calculations was 1.00E-3 cm\(^3\) and a surface area \((SA)\) of 0.023 cm\(^2\)

| Parameter                          | Predictions        |
|------------------------------------|---------------------|
| Donor conc. Cd (µM)                | 9.05                |
| Receiver conc. Cr (µM)             | 1.23                |
| Receiver conc. + inhibitor Cr (µM)| 0.13                |
| \(P_{app}\) (cm/min)               | 0.00599             |
| \(P_{app}\) + inhibitor (cm/min)   | 0.00063             |
| \(PS_{active} + PS_{passive}\) (µL/min) | 0.14               |
| \(P_{passive}\) (µL/min)          | 0.014               |
| \(P_{active}\) (µL/min)           | 0.12                |

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