Article title: Effects of renal impairment on the pharmacokinetics, safety, and tolerability of pudexacianinium (ASP5354) after IV administration: a mechanistic exploration

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Supplementary Information

In vitro study methods

Chemicals and materials

Pudexacianinium and pudexacianinium labeled with ^{14}C ([^{14}C]pudexacianinium) were provided by Astellas Pharma Inc. (Tokyo, Japan). Medium 199, verapamil, probenecid, quinidine anhydrous, cimetidine, and bovine serum albumin were provided by Sigma-Aldrich (St. Louis, MO, USA). Hanks' balanced salts solution (HBSS), Dulbecco's Modified Eagle Medium (DMEM), and fetal bovine serum (FBS) were provided by Thermo Fisher Scientific (Waltham, MA, USA). [^{3}H]digoxin, [^{3}H]prazosin, [^{14}C]mannitol, [^{14}C]caffeine, and estrone sulfate ammonium salt [6,7-$^{3}H(N)$] ([^{3}H]ES) were provided by PerkinElmer (Waltham, MA, USA). Metformin hydrochloride, [biguanidine-^{14}C] ([^{14}C]metformin) was provided by Curachem (Gyeonggi, South Korea). 4-Aminohippuric acid [glycyl-2-^{3}H] ([^{3}H]PAH) was provided by American Radiolabeled Chemicals (St. Louis, MO, USA). Ko143 was provided by Bio-Techne (Minneapolis, MN, USA).

Cells

P-gp- or BCRP-expressing cells (porcine kidney epithelial [LLC-PK1] cells transfected with a vector including human P-gp or BCRP cDNA) and control cells (LLC-PK1 cells transfected with an empty vector) were used under a sublicense from BD Biosciences (San Jose, CA, USA).

The P-gp- or BCRP-expressing cells and the control cells were cultured in Medium 199 containing 10% FBS with antibiotic-antimycotics at 37°C in an atmosphere of 5% CO_2. Before starting the experiments, the P-gp- and BCRP-expressing cells and the control cells were seeded in plates (culture insert, catalog numbers 353096 [P-gp] and 353495 [BCRP], and cell culture insert companion plate [catalog number 353504], Corning, NY, USA) and incubated in a CO_2 incubator (37°C; 5% CO_2).

Human embryonic kidney (HEK293) cells expressing OAT1, OAT3, OCT2, MATE1, or MATE2-K (HEK293 cells transfected with vector containing human OAT1, OAT3, OCT2, MATE1, or MATE2-K cDNA) and control cells (HEK293 cells transfected with vector only) were established at Sekisui Medical Co., Ltd. (Ibaraki, Japan). The cells were cultured in DMEM containing 10% FBS with antibiotic-antimycotics and L-glutamine at 37°C in an atmosphere of 5% CO_2. Before starting the experiments, the HEK293 cells expressing OAT1, OAT3, OCT2, MATE1, or MATE2-K and the control cells were seeded in plates coated with collagen I and incubated in a CO_2 incubator (37°C; 5% CO_2).

Transport of [^{14}C]pudexacianinium by human transporters (LLC-PK1 cells)

The medium was removed from the culture insert and plate and replaced with HBSS (pH 7.4) containing 0.2% DMSO or an inhibitor solution (30 μmol/L verapamil [P-gp] or 1 μmol/L Ko143 [BCRP]

in HBSS, pH 7.4, containing 0.2% DMSO), and the cells were preincubated at 37°C for 11 hours. The solution in the apical side or the basal side (donor side) was replaced with each of the test solutions: Group A, [^{14}C]pudexacianinium at 1 μmol/L or 10 μmol/L; Group B, [^{14}C]pudexacianinium at 1 μmol/L or 10 μmol/L and verapamil at 30 μmol/L (P-gp) or Ko143 at 1 μmol/L (BCRP); Group C, [^{3}H]digoxin at 1 μmol/L (P-gp) or [^{3}H]prazosin at 0.01 μmol/L (BCRP); Group D, [^{3}H]digoxin at 1 μmol/L and verapamil at 30 μmol/L (P-gp) or [^{3}H]prazosin at 0.01 μmol/L and Ko143 at 1 μmol/L (BCRP); Group E, [^{14}C]mannitol at 10 μmol/L; Group F, [^{14}C]caffeine at 10 μmol/L. The solution in the other side (receiver side) was replaced with HBSS (pH 7.4) containing 0.2% DMSO or the inhibitor solution. The cells were then incubated at 37°C. After incubation for the designated time(s) ([^{14}C]pudexacianinium: 30 minutes 1 hour, and 2 hours; [^{3}H]digoxin, [^{14}C]mannitol, and [^{14}C]caffeine: 2 hours; and [^{3}H]prazosin: 1 hour), an aliquot was collected from the receiver side when [^{14}C]pudexacianinium, [^{3}H]digoxin, [^{3}H]prazosin, [^{14}C]mannitol, or [^{14}C]caffeine was spiked to the donor side as the analytical sample of the transcellular transport. For Groups A and B, to compensate for the volumes sampled after incubation for 30 minutes and 1 hour, an aliquot of HBSS (pH 7.4) containing 0.2% DMSO or the inhibitor solution pre-warmed at 37°C was added immediately after sampling and used as the analytical sample.

Each analytical sample was mixed with Emulsifier-Safe (PerkinElmer, Waltham, MA, USA), and the radioactivity present in the sample was measured using a liquid scintillation counter (LSC; 2500TR, 3100TR, or 3110TR [PerkinElmer, Waltham, MA USA]). To determine the initial radioactivity (C_0), the analytical sample was collected into a vial before incubation. The collected sample was mixed with Emulsifier-Safe and the radioactivity in the solution was measured using an LSC.

The amount of pudexacianinium transported across the control cell monolayer and the P-gp- or BCRP-expressing cell monolayer was determined according to the following equation:

P_{app} [cm/s] = [dQ/dt]/A/C_0

In this equation, dQ/dt is the transport rate (dpm/s); A is the membrane area (cm^2); and C_0 is the initial concentration (dpm/mL).

The ratio of the permeability coefficients was calculated from the basal to apical P_{app} and the apical to basal P_{app} according to the following equation:

P_{app} ratio = Basal to apical P_{app} (cm/s)／Apical to basal P_{app} (cm/s)

Furthermore, the ratios of the P_{app} ratio in P-gp- or BCRP-expressing cells to the P_{app} ratio in the control cells were calculated as the corrected P_{app} ratio according to the following equation:

Corrected P_{app} ratio = P_{app} ratio of the P-gp- or BCRP-expressing cells／P_{app} ratio of the control cells

Transport of [^{14}C]pudexacianinium by human transporters (HEK293 cells)

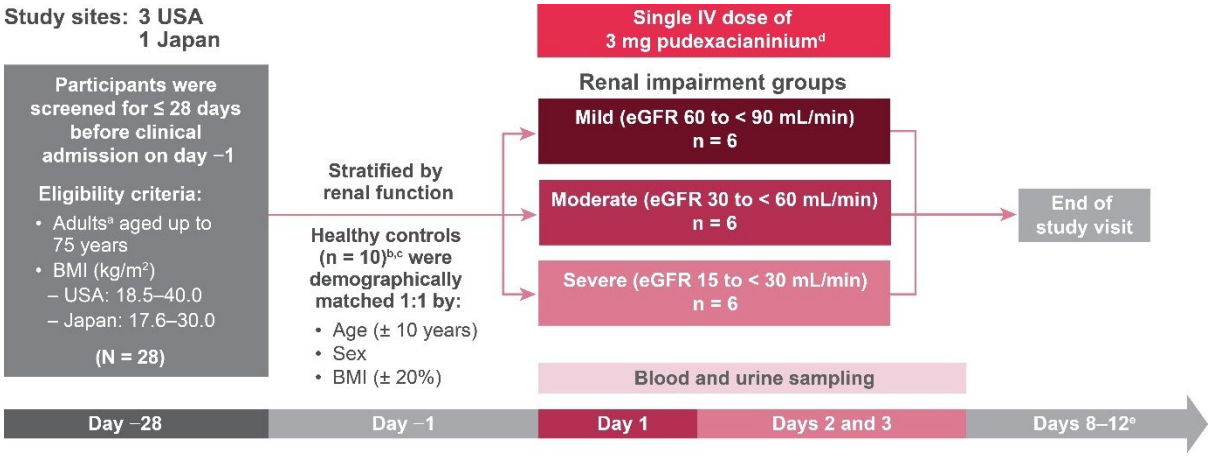
The medium was removed from the plate seeded with transporter-expressing or control cells and replaced with HBSS. HBSS was removed and replaced with HBSS containing 0.2% DMSO or an inhibitor solution (probenecid at 100 μmol/L [OAT1 and OAT3], quinidine at 300 μmol/L [OCT2], cimetidine at 10 μmol/L [MATE1], or cimetidine at 100 μmol/L [MATE2-K] in HBSS containing 0.2% DMSO) and the plate was preincubated at 37°C for 15 minutes. After preincubation, the buffer was replaced with test solution: Group A, [^{14}C]pudexacianinium at 1 μmol/L or 10 μmol/L; Group B, [^{14}C]pudexacianinium at 1 μmol/L or 10 μmol/L and probenecid at 100 μmol/L (OAT1 and OAT3), quinidine at 300 μmol/L (OCT2), cimetidine at 10 μmol/L (MATE1), or cimetidine at 100 μmol/L (MATE2-K); Group C, [^{3}H]PAH at 1 μmol/L (OAT1), [^{3}H]ES at 0.05 μmol/L (OAT3), or [^{14}C]metformin at 10 μmol/L (OCT2, MATE1, and MATE2-K); Group D, [^{14}C]metformin at 10 μmol/L and probenecid at 100 μmol/L (OAT1 and OAT3), quinidine at 300 μmol/L (OCT2), cimetidine at 10 μmol/L (MATE1), or cimetidine at 100 μmol/L (MATE2-K). Each resulting mixture was incubated at 37°C for the designated time(s) ([^{14}C]pudexacianinium: 1, 2, 5, and 10 minutes; [^{3}H]PAH for OAT1: 0.5 minutes; [^{3}H]ES and [^{14}C]metformin for OAT3 and OCT2: 2 minutes; and [^{14}C]metformin for MATE1 and MATE2-K: 5 minutes). After incubation, each solution was removed, and the cells were washed once with ice-cold phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin and then twice with ice-cold PBS. The PBS was removed, and the cells were dissolved in sodium hydroxide (0.1 mol/L).

The resulting cell solution was mixed by pipetting, and the mixed solution was collected into a vial, mixed with Emulsifier-Safe, and analyzed for radioactivity using a LSC. To determine the initial radioactivity, the cell solution was collected into a vial before incubation and mixed with Emulsifier-Safe, and the radioactivity in the solution was measured using an LSC. The incubation was performed in triplicate.

Protein content was measured using the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's recommendations. The cleared volume (μL/mg protein) at each incubation time was calculated as follows: uptake amount of pudexacianinium (dpm/well)／(initial concentration [dpm/μL] × protein amount [mg/well]).

Supplementary figures

Fig. S1: Study design schematic



[a]Aged ≥ 18 years in the USA or ≥ 20 years in Japan.

[b]Participants with normal renal function were defined by eGFR ≥ 90 mL/min.

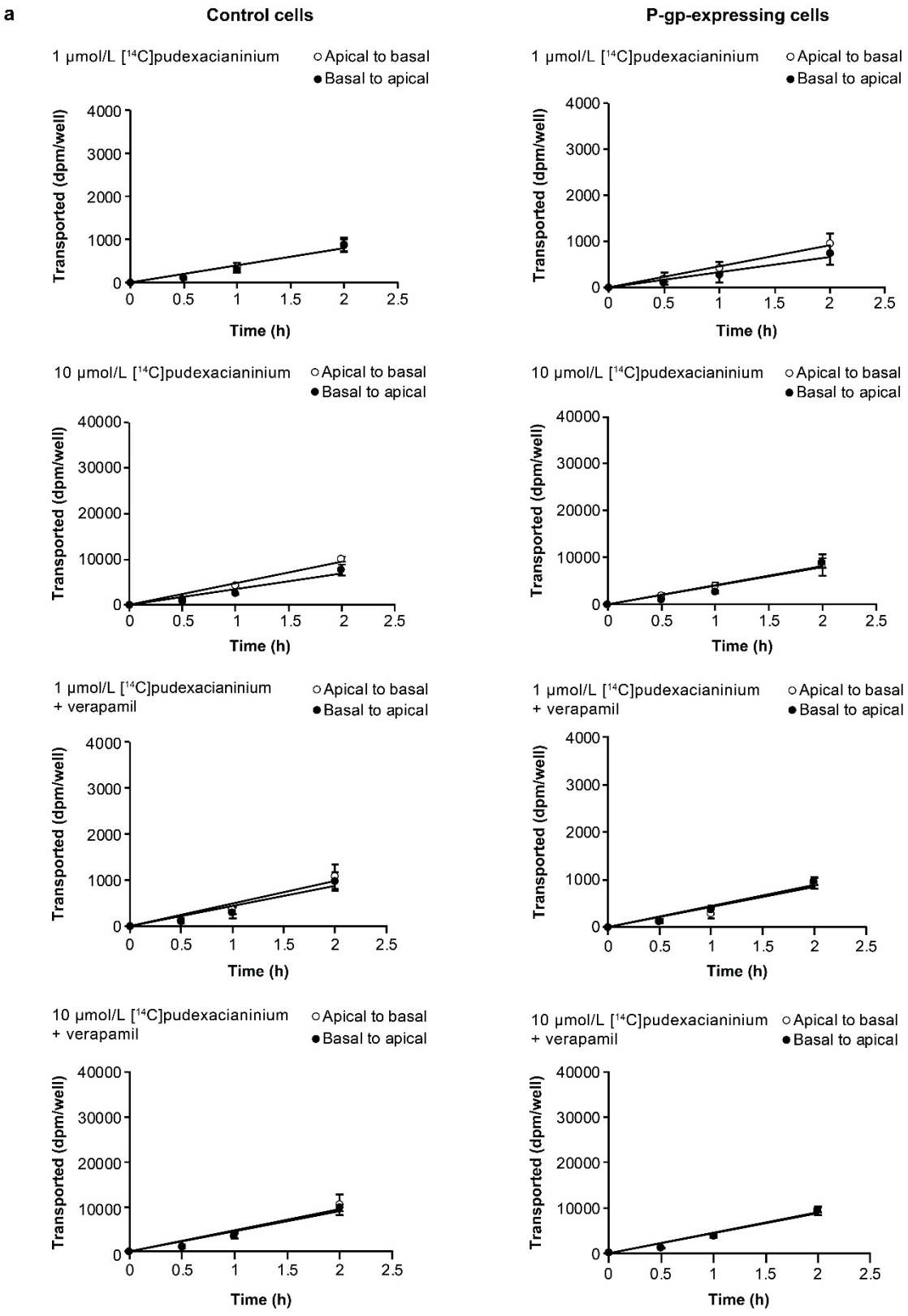
[c]Control participants could be demographically matched with multiple participants with renal impairment provided matched participants were classed in different groups of renal impairment.

[d]On Day 1, pudexacianinium was administered with 240 mL water to fasted participants (e.g., no calorie intake permitted from ≥ 10 hours before dosing through 4 hours after dosing).

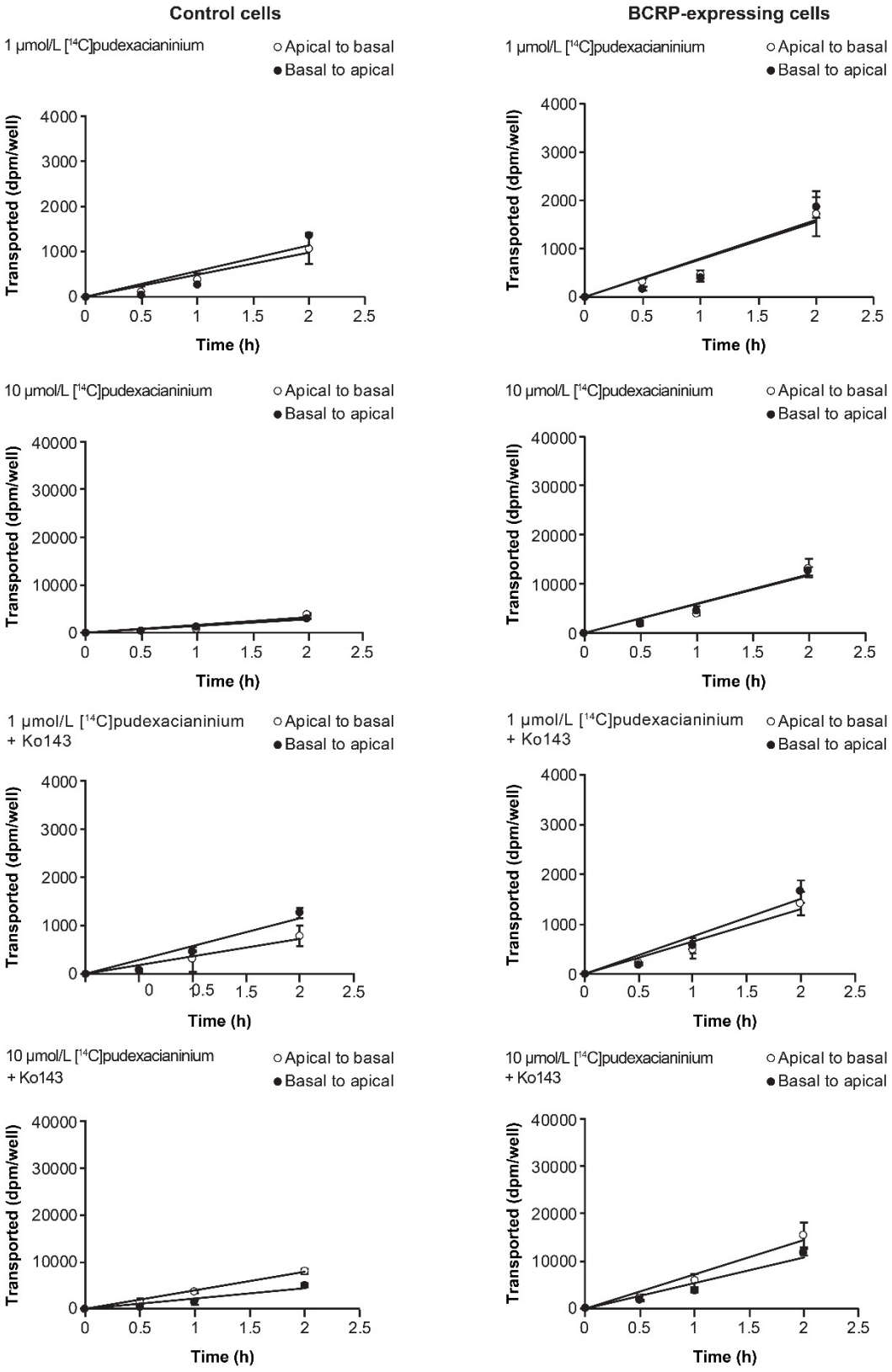
[e]The end-of-study visit took place 5–9 days after the last pharmacokinetic sample was collected.

BMI, body mass index; eGFR, estimated glomerular filtration rate; IV, intravenous.

Fig. S2: Transcellular transport of [^{14}C]pudexacianinium (1 µmol/L and 10 µmol/L) across control cells and LLC-PK1 cell monolayers expressing (A) P-gp and (B) BCRP

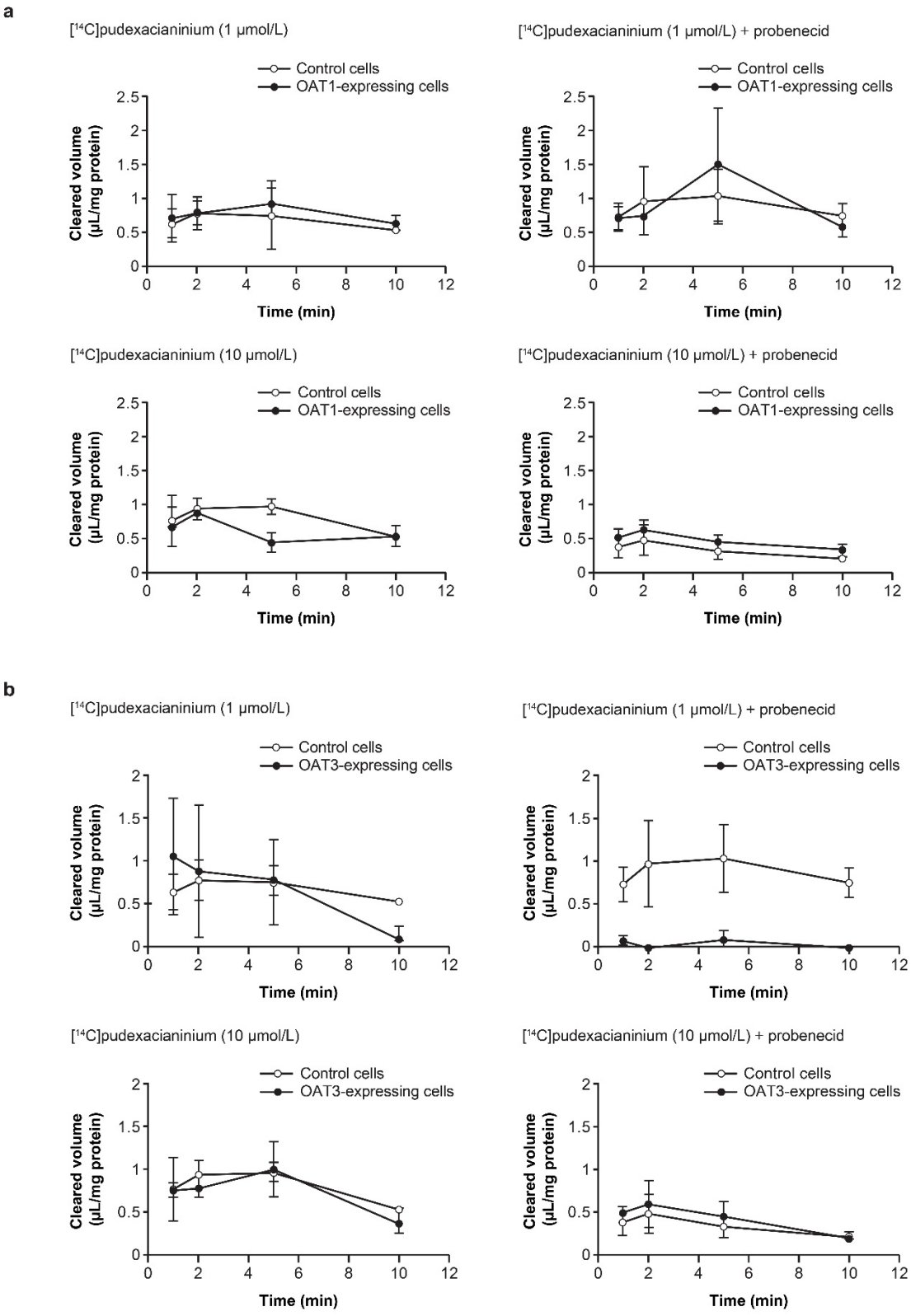


b

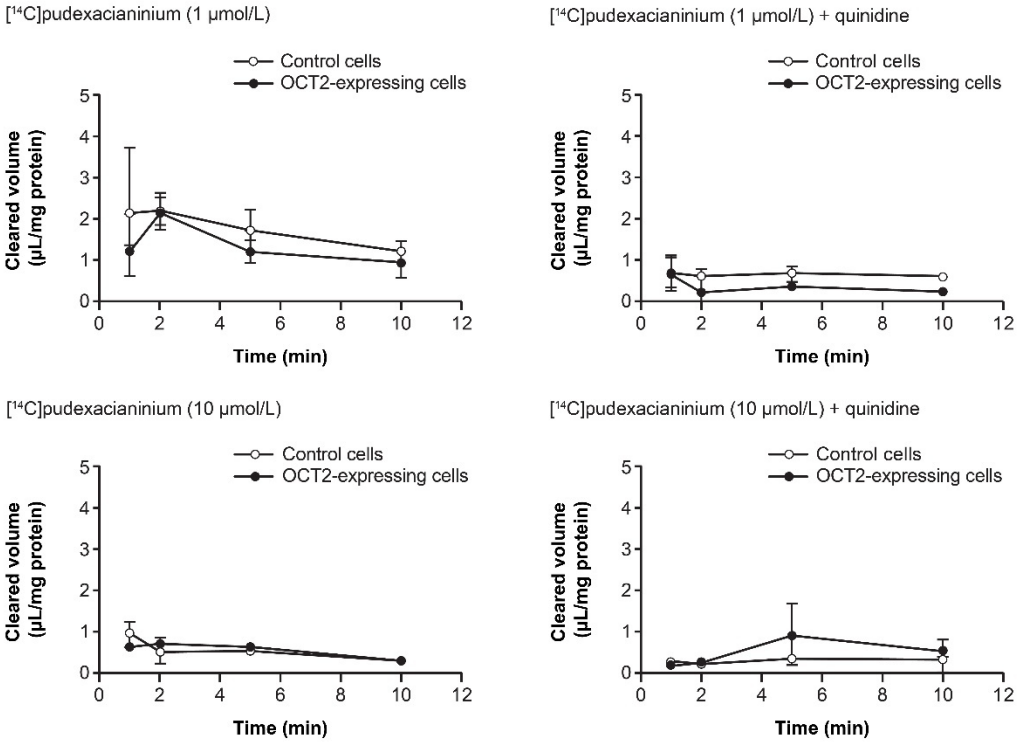


Each point represents the mean ± standard deviation of triplicate determinations.

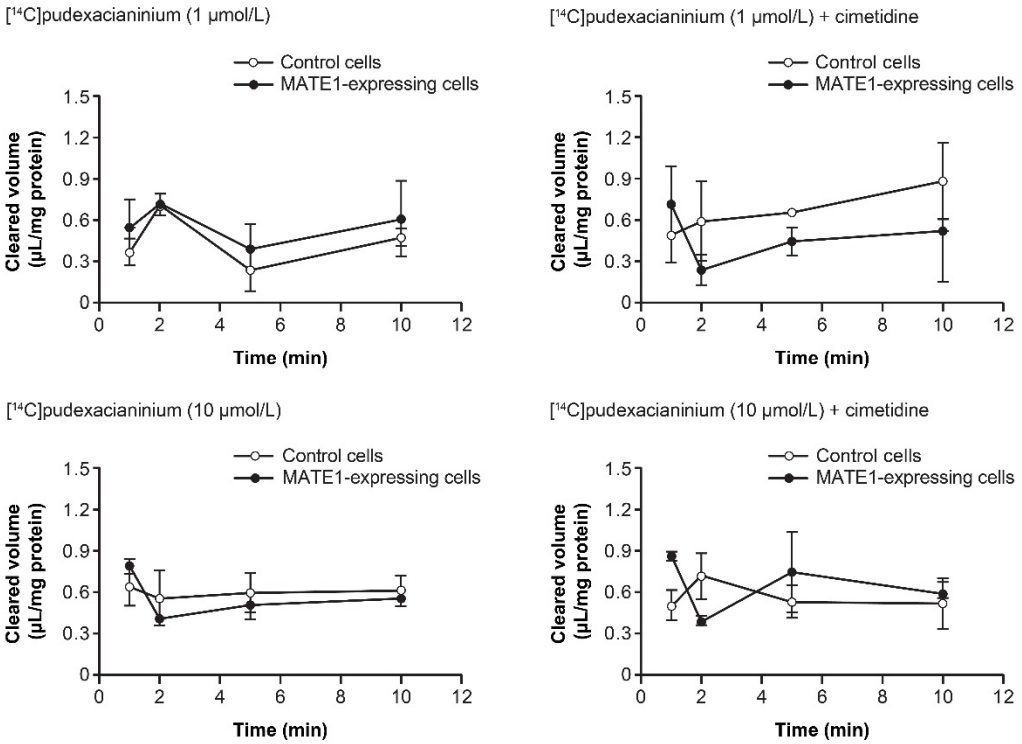
Fig. S3: Uptake of [^{14}C]pudexacianinium (1 µmol/L and 10 µmol/L) into control cells and cells expressing (A) OAT1, (B) OAT3, (C) OCT2, (D) MATE1, and (E) MATE2-K



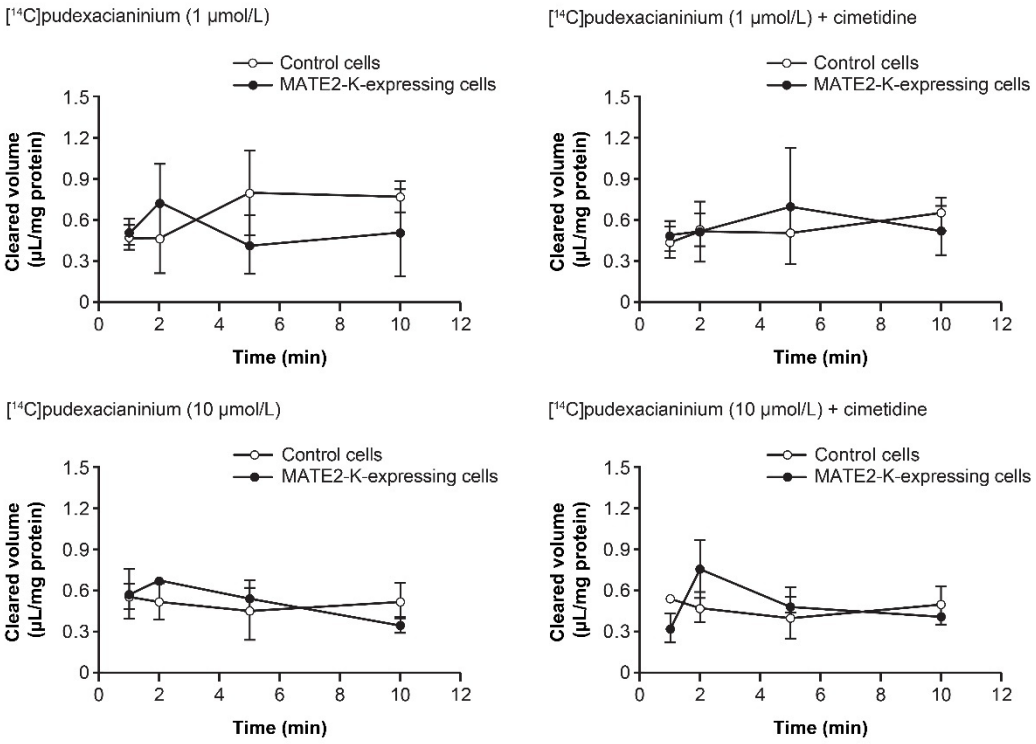
c


[14C]pudexacianinium (1 µmol/L)
Control cells
OCT2-expressing cells
Cleared volume (µL/mg protein)
Time (min)
[14C]pudexacianinium (1 µmol/L) + quinidine
Control cells
OCT2-expressing cells
Cleared volume (µL/mg protein)
Time (min)
[14C]pudexacianinium (10 µmol/L)
Control cells
OCT2-expressing cells
Cleared volume (µL/mg protein)
Time (min)
[14C]pudexacianinium (10 µmol/L) + quinidine
Control cells
OCT2-expressing cells
Cleared volume (µL/mg protein)
Time (min)

d

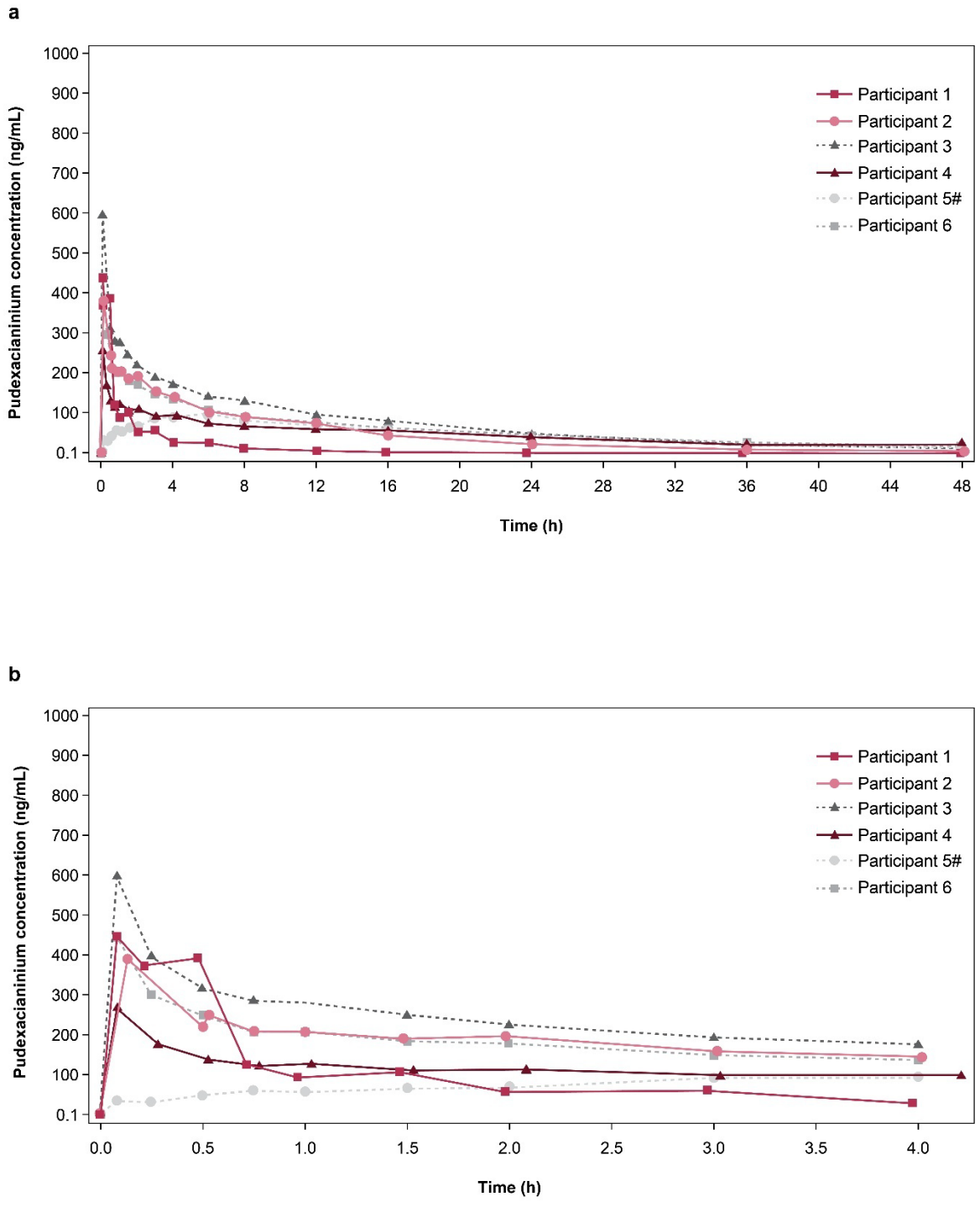

[14C]pudexacianinium (1 µmol/L)
Control cells
MATE1-expressing cells
Cleared volume (µL/mg protein)
Time (min)
[14C]pudexacianinium (1 µmol/L) + cimetidine
Control cells
MATE1-expressing cells
Cleared volume (µL/mg protein)
Time (min)
[14C]pudexacianinium (10 µmol/L)
Control cells
MATE1-expressing cells
Cleared volume (µL/mg protein)
Time (min)
[14C]pudexacianinium (10 µmol/L) + cimetidine
Control cells
MATE1-expressing cells
Cleared volume (µL/mg protein)
Time (min)

e



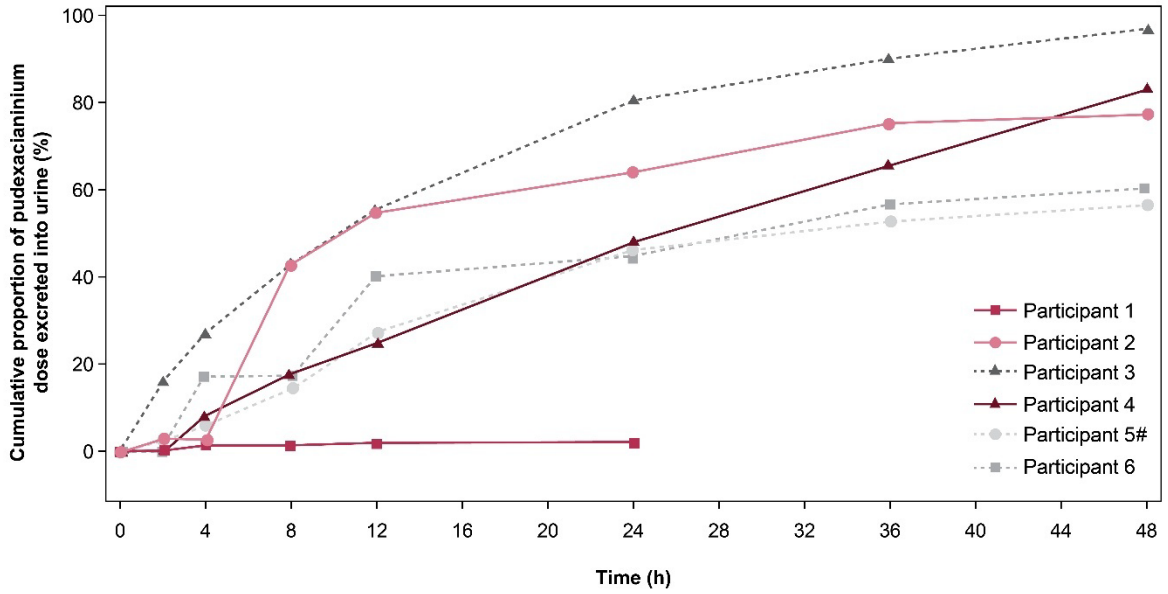
Each point represents the mean ± standard deviation of triplicate determinations.

Fig. S4: Plasma concentration profiles of pudexacianinium for individual participants with severe renal impairment over periods of (A) up to 48 hours and (B) up to 4 hours



#Participant with suspected extravascular administration, who was excluded from the pharmacokinetic analysis set.

Fig. S5: Cumulative proportion of pudexacianinium dose excreted into urine (for individual participants with severe renal impairment)



#Participant with suspected extravascular administration, who was excluded from the pharmacokinetic analysis set.