

Lipid Conjugates of Cidofovir and Tenofovir, CMX001 and CMX157, Are Not Substrates of Human Organic Anion Transporters hOAT1 and hOAT3

TK Tippin, BM Lampert, GR Painter and ER Lanier
Chimerix Inc., Durham, NC, USA

OBJECTIVE

To determine whether CMX001 and CMX157, the lipid conjugates of cidofovir and tenofovir, are substrates of human Organic Anion Transporter 1 (hOAT1) and hOAT3 using cell-based methods.

INTRODUCTION

Cidofovir (CDV) and Tenofovir (TFV) are polar, acyclic nucleoside phosphonates that are FDA-approved as Vistide® (cidofovir injection) for the treatment of cytomegalovirus retinitis, and as Viread® (tenofovir disoproxil) for the treatment of HIV. CMX001 [3-(hexadecyloxy)propyl hydrogen ((S)-1-(4-amino-2-oxopyrimidin-1(2H)-yl)-3-hydroxypropan-2-yl)oxy)methyl phosphonate], and CMX157 [3-(hexadecyloxy)propyl hydrogen ((R)-1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl phosphonate], the lipid conjugates of CDV and TFV, respectively, have demonstrated increased potency in cell based assays relative to CDV and TFV, respectively, and in the case of CMX001 has proven effective in vivo in animals after oral administration (1). Importantly, no signs of nephrotoxicity have been observed in animal toxicology studies or in human clinical trials to date after oral administration of CMX001 or CMX157 (2), a distinct advantage compared to CDV, and to a lesser extent TFV, both of which are known to accumulate in kidney proximal tubule cells through their selective uptake by organic anion transporter 1 (OAT1) and OAT3 (3,4). The purpose of this study was to explore whether CMX001 and CMX157 are substrates of human OAT1 and OAT3.

METHODS

Cellular uptake. MDCK-II cells were grown on semi-permeable filters (1 μ M, polyethylene terephthalate (PET), Millipore), and transiently transfected with hOAT1, hOAT3 or vector only using a proprietary technique (Optivia Biotechnology). Compounds (CMX001, CMX157, TFV at 5 μ M and CDV at 25 μ M), with or without probenecid, an OAT inhibitor (100 μ M), in the presence or absence of 20% human serum, were added to the basolateral side of the cell monolayer (n=4 replicates/condition). After a 5 min incubation period, drug solutions were removed from cells, and the cells were rinsed, extracted with 50% acetonitrile, and analyzed by LC/MS/MS. Net OAT-mediated uptake was determined from total uptake in OAT-expressing cells minus uptake in vector-treated control cells. Minimum established acceptance criteria for hOAT1 activity was >0.71 pmol/min/cm² for p-aminohippurate (PAH), with > 70% inhibition in the presence of probenecid; and for hOAT3 activity was >0.62 pmol/min/cm² for estrone-3-sulfate, with > 90% inhibition in the presence of probenecid. Statistical significance (p<0.05) of the cellular uptake of compounds in transfected vs. control, \pm probenecid or \pm 20% serum was assessed using an unpaired t-test. Statistical analysis of multiple parameters was performed using analysis of variance (ANOVA).

LC/MS/MS. Cell extracts were analyzed (Integrated Analytical Solutions) for CMX001 or CMX157 using a Peake Scientific Polymeric SDB (10 x 2 mm) column with an initial mobile phase of 90% water/8% acetonitrile/2% tetrahydrofuran (containing 0.1% (v/v) formic acid), held constant for 0.25 min, and then changed to 80% acetonitrile/20% tetrahydrofuran (containing 0.1% formic acid, v/v) over a 1.25 min linear gradient. The flow rate was 0.8 mL/min. Total ion chromatograms and MS-MS spectra of ions (m/z 562.4/261.9 and 570.5/269.9, respectively) were obtained using an API 3000 mass spectrometer using electrospray ionization (400°C) in positive ion mode. For CDV, a Peake Scientific HILIC SM (30 x 2.1 mm) column with an initial mobile phase of 95% acetonitrile/5% water (containing 0.1% (v/v) formic acid), held constant for 0.25 min, and then changed to 5% acetonitrile/95% water (containing 0.1% (v/v) formic acid) over a 1.25 min linear gradient was used. The flow rate was 0.8 mL/min, and ions (m/z 280.2/86.0) were detected as described above. For TFV, a Peake Scientific Titan C18 (20 x 2 mm) column with an initial mobile phase of 0.1% heptafluorobutyric acid (HFBA) in water, held constant for 0.25 min, and then changed to 95% methanol (containing 0.1% (v/v) formic acid)/5% water (containing 0.1% HFBA) over a 1.25 min linear gradient was used. The flow rate was 0.8 mL/min, and ions (m/z 288.2/176.3) were detected as described above.

hOAT-Mediated Uptake of CMX001 and CDV

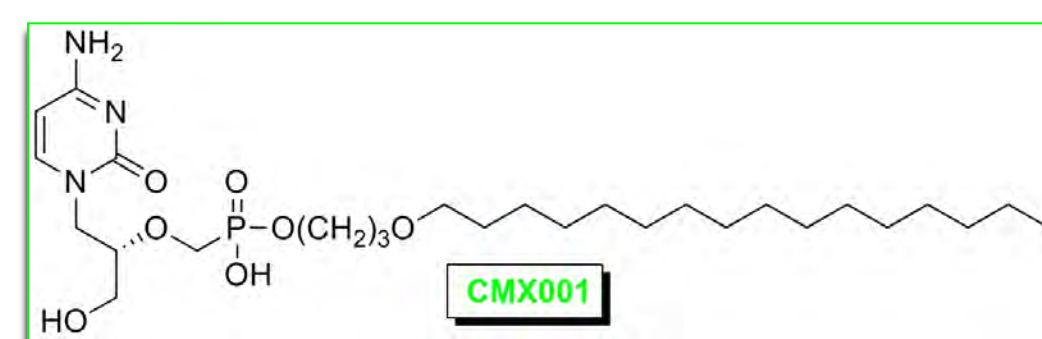


Figure 1. Net hOAT1 and hOAT3 mediated **CMX001** uptake in the absence or presence of 100 μ M probenecid

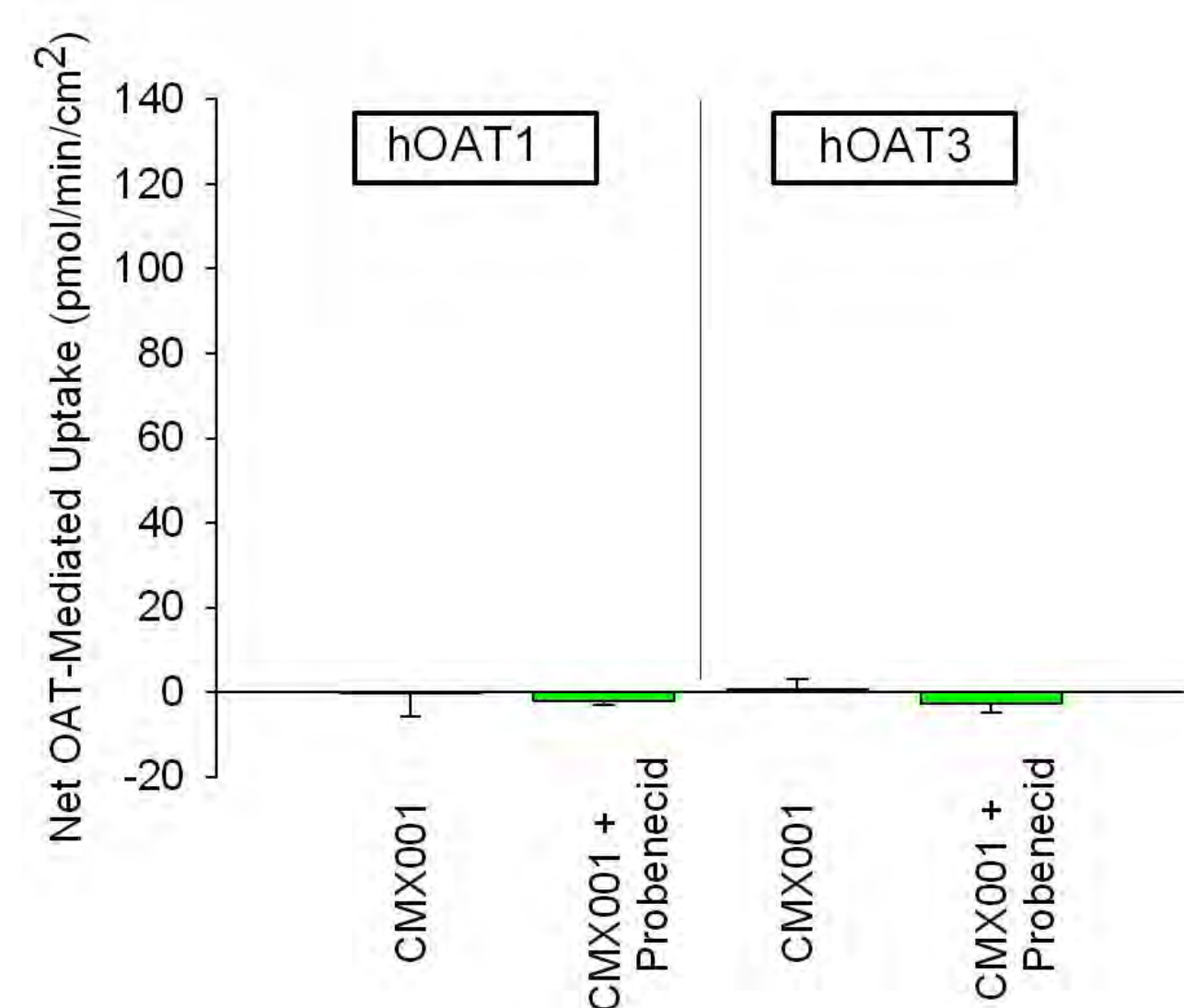
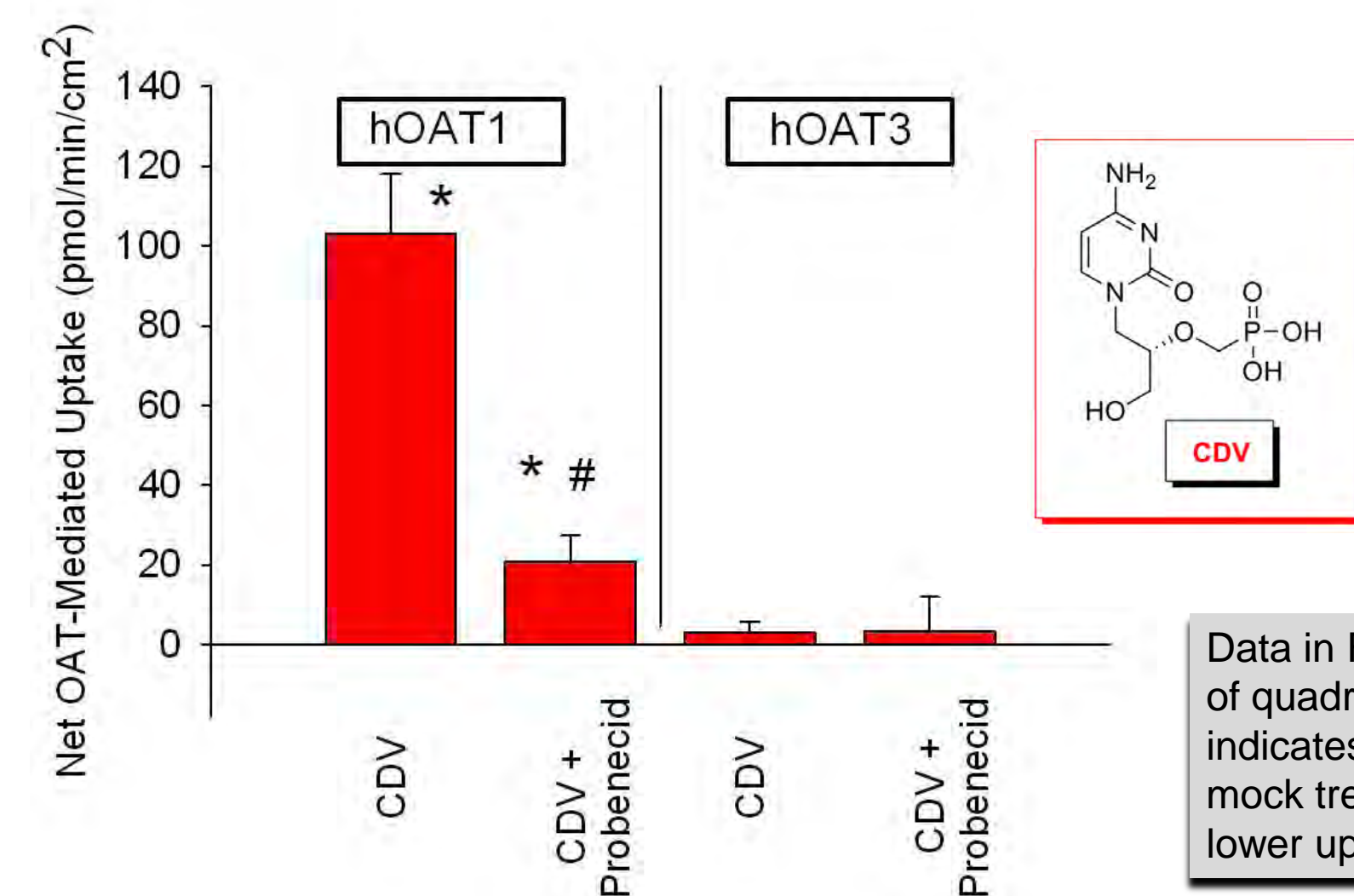


Figure 2. Net hOAT1 and hOAT3 mediated **CDV** uptake in the absence or presence of 100 μ M probenecid



Data in Figures are mean and standard deviation of quadruplicate samples. An asterisk (*) indicates statistical significance (p < 0.05) vs. mock treated cells; (#) indicates significantly lower uptake in the presence of probenecid.

□ Net uptake of CMX001 (5 μ M) was not enhanced in hOAT1 and hOAT3 expressing cells, nor was uptake decreased in the presence of OAT inhibitor probenecid (Figure 1).

□ Net uptake of CDV (25 μ M) was enhanced in hOAT1 expressing cells, and decreased in the presence of OAT inhibitor probenecid, but was not enhanced in hOAT3 expressing cells (Figure 2).

RESULTS

hOAT-Mediated Uptake of CMX157 and TFV

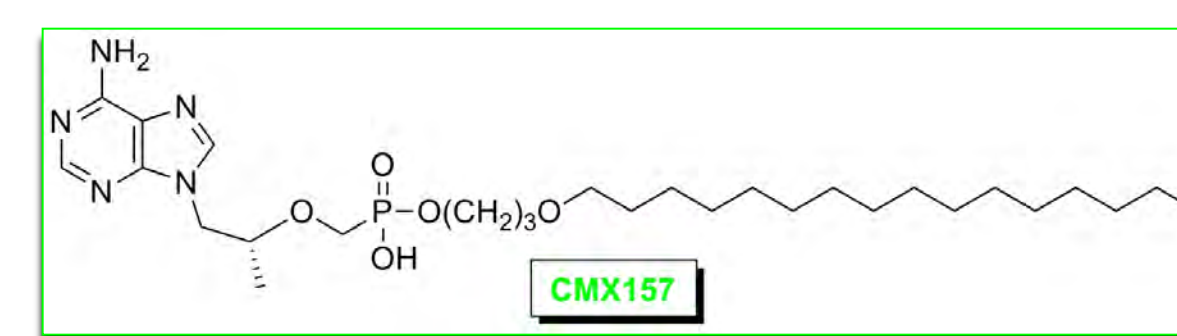


Figure 3. Net hOAT1 and hOAT3 mediated **CMX157** uptake in the absence or presence of 100 μ M probenecid

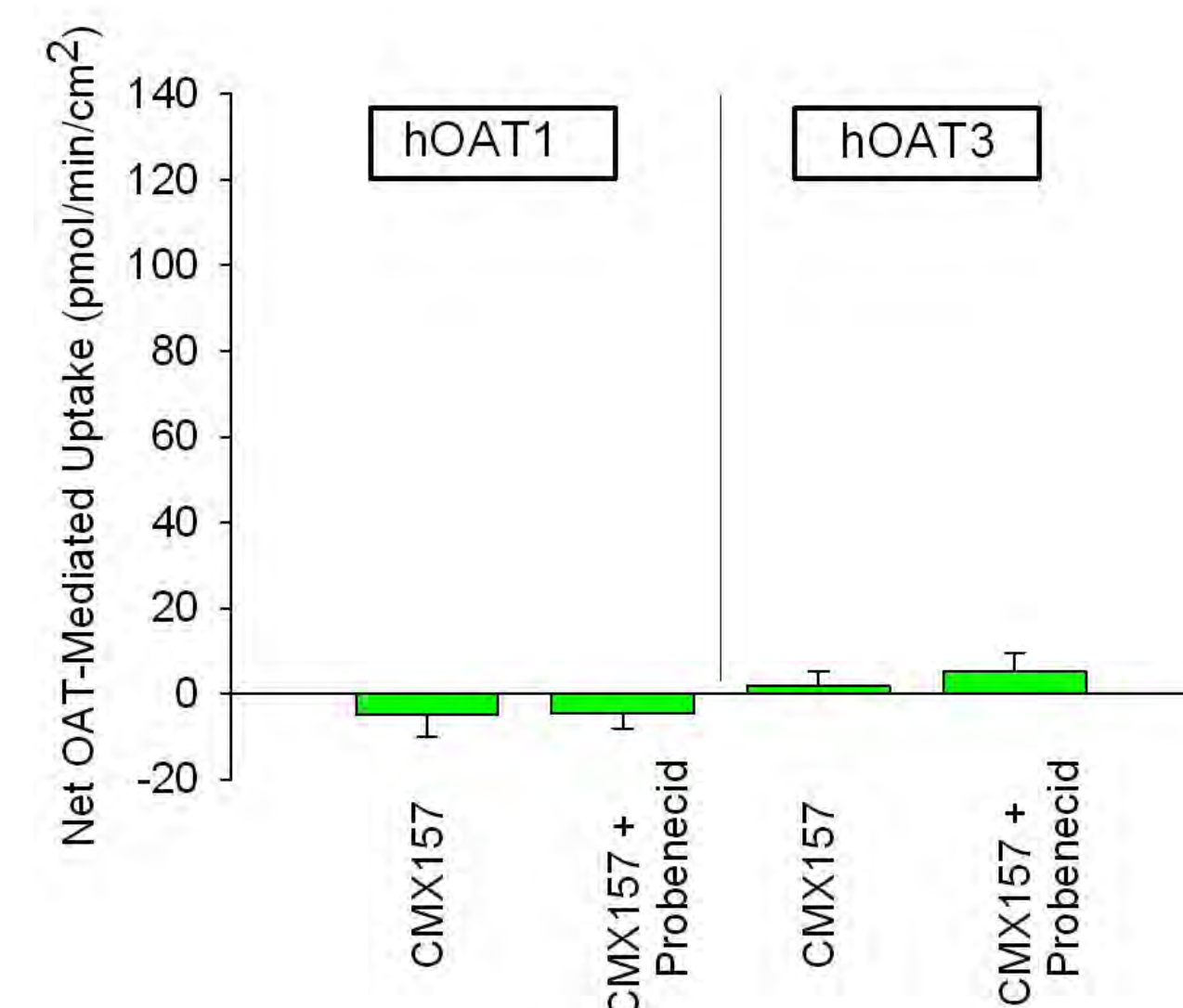
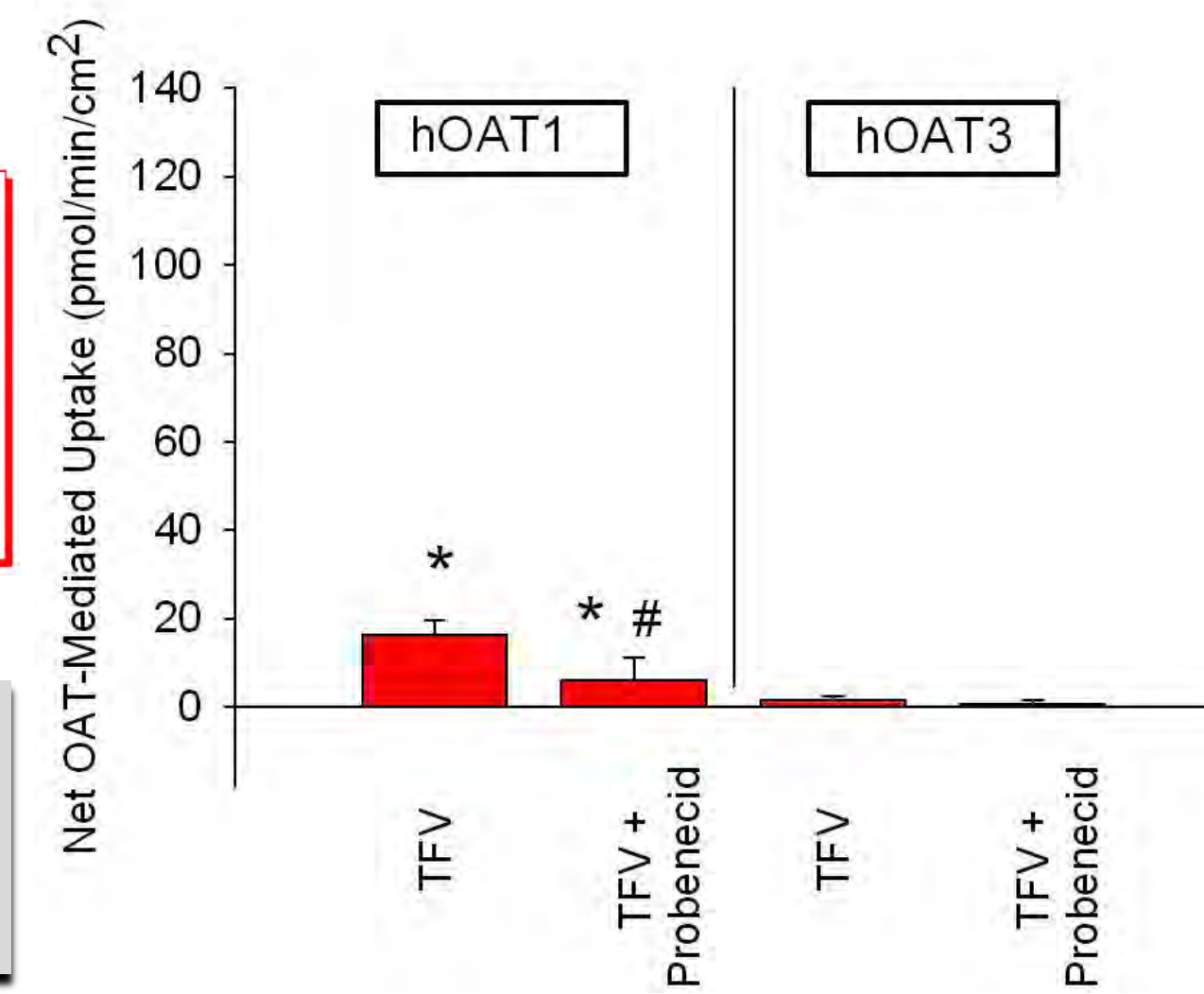


Figure 4. Net hOAT1 and hOAT3 mediated **TFV** uptake in the absence or presence of 100 μ M probenecid



□ Net uptake of CMX157 (5 μ M) was not enhanced in hOAT1 and hOAT3 expressing cells, nor was uptake decreased in the presence of OAT inhibitor probenecid (Figure 3).

□ Net uptake of TFV (5 μ M) was enhanced in hOAT1 expressing cells, and decreased in the presence of OAT inhibitor probenecid, but was not enhanced in hOAT3 expressing cells (Figure 4).

Table 1. Effect of Serum on hOAT1-Mediated Uptake of CMX001 and CMX157

Compound (Concentration)	Treatment	Uptake (Transporter)	Uptake (Control)	Net Transporter Uptake
CMX001 (5 μ M)	Buffer	15.3 5.4	15.6 4.3	-0.3 5.4
	20% serum	BLOQ	0.6	ND
CDV (25 μ M)	Buffer	117 15	13.8 5.5	104 15
	20% serum	116 22	13.5 8.0	102 22
CMX157 (5 μ M)	Buffer	11.0 5.3	15.8 8.3	-4.9 5.3
	20% serum	1.7 0.4	1.7 0.4	0.0 0.4
TFV (5 μ M)	Buffer	19.7 3.3	3.4 2.4	16.3 3.3
	20% serum	18.9 4.4	8.8 4.7	10.2 4.4

Values are mean \pm std deviation in units, pmol/min/cm²; BLOQ, below limit of quantitation; ND, not determined.

□ In the presence of 20% serum, uptake of CMX001 and CMX157 in hOAT1-expressing cells and control cells was substantially reduced, indicating that passive uptake of both compounds is reduced by binding to serum protein.

DISCUSSION

- Neither CMX001 nor CMX157 uptake is enhanced in vitro in cells expressing hOAT1 or hOAT3 compared to control cells that do not express these transporters under the conditions used in this study.
- In contrast, uptake of CDV and TFV was enhanced in vitro in cells expressing hOAT1, which confirms literature reports of efficient uptake of CDV and TFV by hOAT1. Despite demonstrated functional uptake of hOAT3 substrate estrone-3-sulfate, no hOAT3-mediated transport of CDV and TFV was observed in this study. This result is consistent with the reported lower uptake efficiency (i.e., > 100-fold lower V_{max}/K_m) for CDV and TFV (5), though some groups (4) have observed enhanced in vitro uptake of CDV and TFV by hOAT3.

CONCLUSIONS

- CMX001 and CMX157 are not substrates for hOAT1 or hOAT3.
- These data combined with the lack of nephrotoxicity observed to date in animals and humans following oral administration of CMX001 and CMX157, suggest that CMX001 and CMX157 have a low potential to cause OAT-mediated nephrotoxicity, a known adverse event following administration of CDV and to a lesser extent TFV.

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