Complementary in vitro tools to investigate renal drug transport

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Introduction

Renal excretion is an important pathway for the elimination of endogenous and xenobiotic substances. A wide range of efflux and uptake transporters are expressed in the renal epithelial cells to regulate the excretion and the reabsorption of various kinds of organic anions, cations, peptides and nucleosides. The purpose of this work to compare two in vitro systems for investigating renal handling: The primary proximal tubule cell (PTC) monolayer assay (established by Dr Colin Brown, Newcastle University, UK) serves as a holistic approach, while double transfected monolayer assays could complete the results gained in PTC model with functional characteristics, such as substrate specificity and transport mechanisms involved in the renal elimination.

Material and Methods

PTCs were isolated from human kidney less than18 hours ex vivo as follows: *1)* kidney decapsulated, cortex dissected and finely chopped; *2)* 2 hour collagenase digest in isolation medium at 37˚C; *3)* the heterogeneous cell population was passed through 40 µM sieve, then separated by density centrifugation; *4)* The tubular cell layer was extracted and cultured on transwell inserts. Unlike other primer kidney cell-based models, the PTC model retains the expressions and the functionality of the kidney transporters:

Gene	Percentage of native kidney expression	
	Filter Support	Plastic
MDR1	65.2 ± 7.1	56.8 ± 9.1
BCRP	31.3 ± 5.5	19.4 ± 7.1
MRP2	31.5 ± 33	20.1 ± 7.8
MRP4	29.3 ± 4.8	22.4 ± 6.5
OAT1	20.6 ± 4.6	11.2 ± 5.3
OAT3	27.8 ± 6.7	9.5 ±4.9
OCT2	39.7 ± 4.3	21.3 ± 6.2
OATP4C1	39.0 ± 2.7	13.5 ± 6.4
SLC2A9	27.7 ± 4.8	29.3 ± 7.6
URAT1	34.6 ± 9.2	16.3 ± 5.6
MATE1	36.4 ± 4.2	14.5 ± 4.8
MATE2K	30.1 ± 8.8	17.2 ± 7.8

Table 1. mRNA level of transporters determined in freshly isolated and plated kidney PTC.

Experiments were performed when human PTC monolayers had a TEER of 80 Ωcm^2 or greater. Uni-directional fluxes of several probe substrates in either the apical to basolateral (J_{A-B}) or basolateral to apical (J_{B-A}) direction were measured.

The **double transfected cell lines** (MDCKII-OCT2/MATE1, MDCKII-OCT2/MATE2-K, MDCKII-OAT1/BCRP and MDCKII-OAT3/BCRP) were developed by SOLVO Biotechnology. The double transfected and the corresponding control cells (parental MDCKII and single transfected transporter expressing MDCKII cells) were plated on 24-transwell plates, and bidirectional transport of several probe substrates were measured after 5 days.

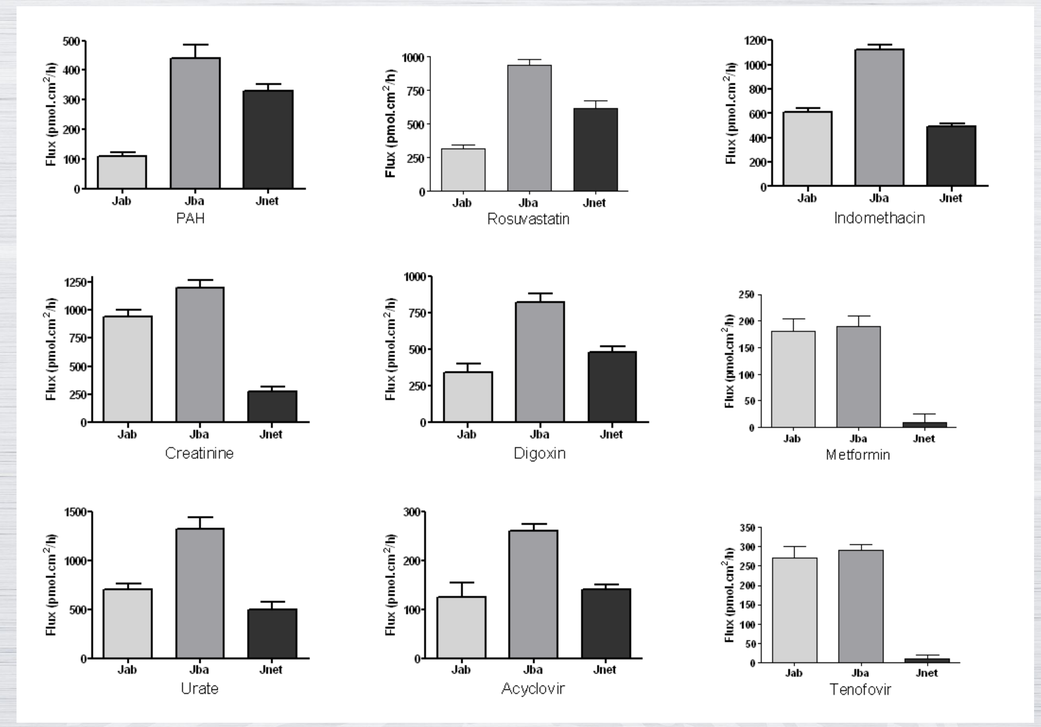

Figure 1. A: Vectorial transport of key prototypic substrates in the proximal tubule cell monolayers

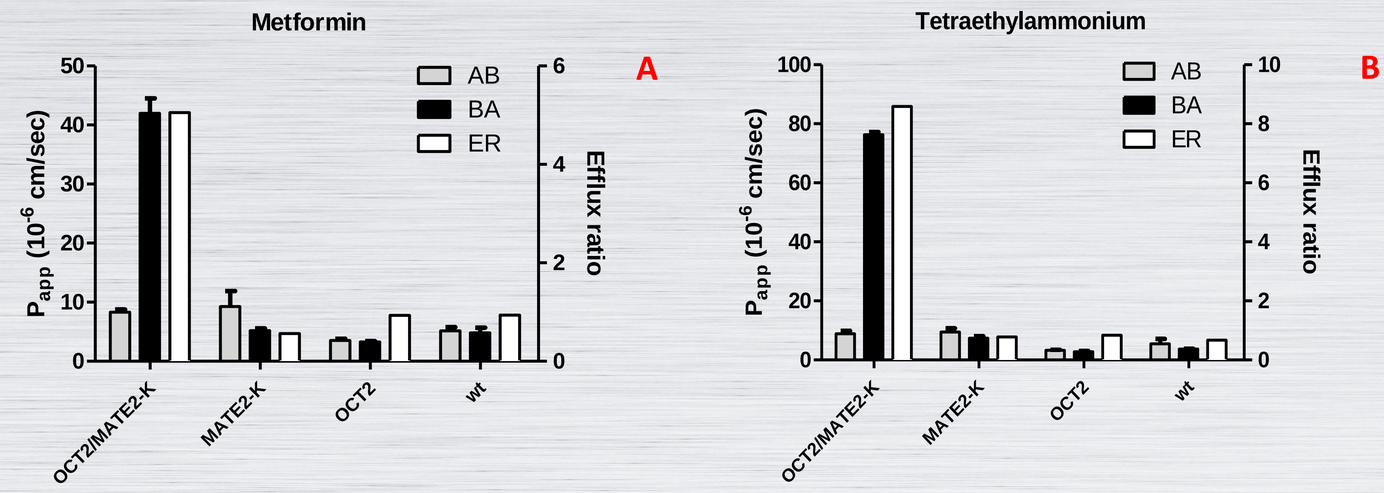

Figure 2. Efflux of metformin (A) and tetraethylammonium (B: TEA) in MDCKII-OCT2/MATE2-K and control cells

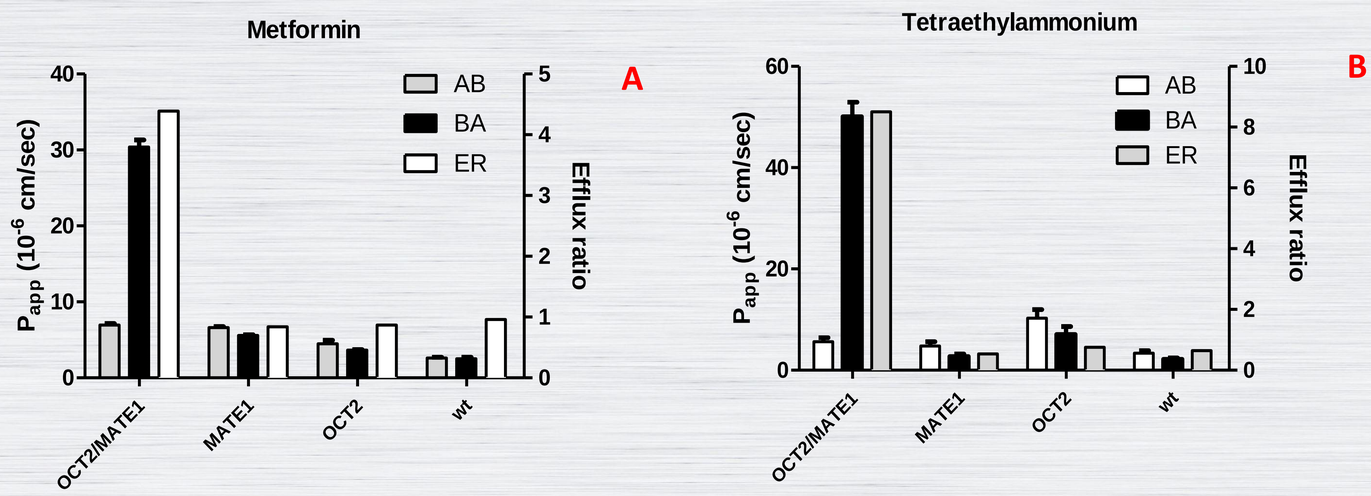

Figure 3. A: Efflux of metformin (A) and and tetraethylammonium (B: TEA) in MDCKII-OCT2/MATE1 and control cells.

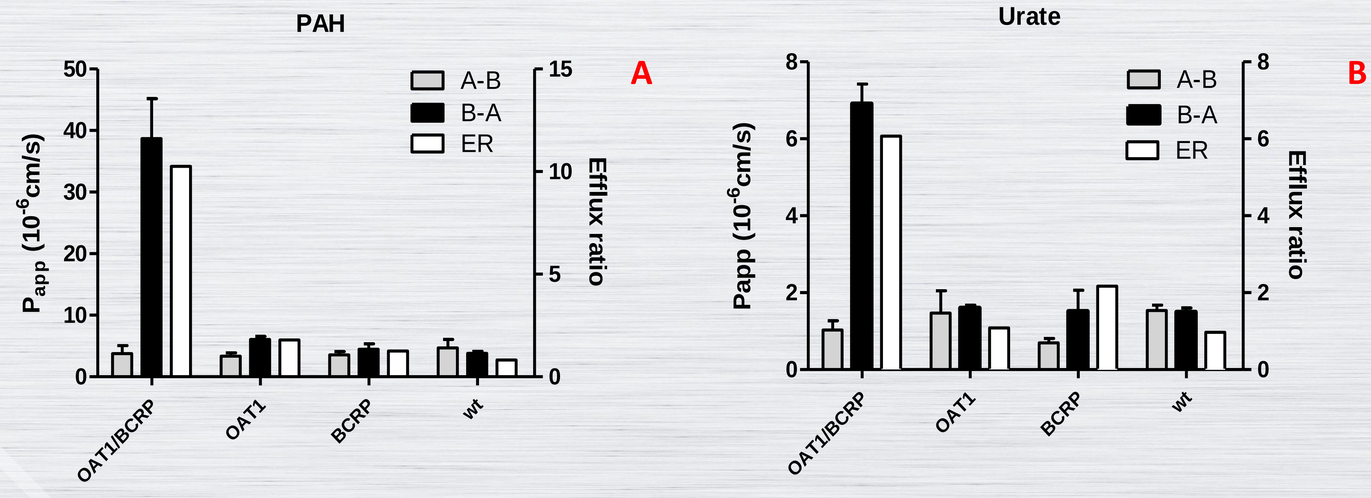

Figure 4. A: Efflux of PAH (A: p-aminohippuric acid) and uric acid (B) in MDCKII-OAT1/BCRP and control cells.

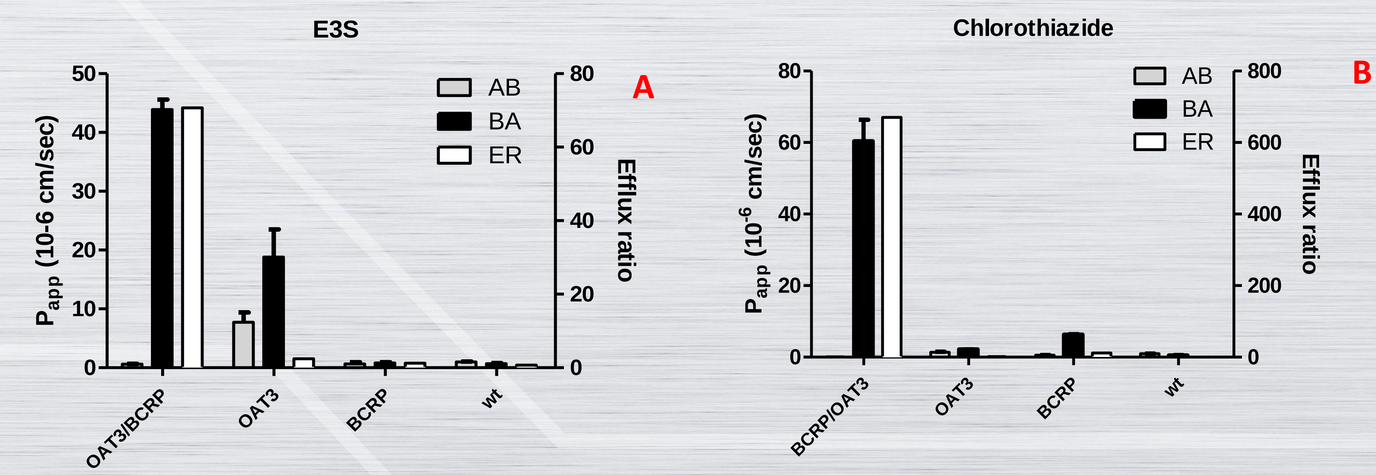

Figure 5. A: Efflux of E3S (A: estrone-3-sulphate) and chlorothiazide in MDCKII-OAT3/BCRP and control cells.

Conclusion

- The vectorial transport of several key prototypic substrate (**Figure 1**) demonstrated that the function of the PTC monolayers are retained to a considerable degree. Consequently, PTC monolayer provides a physiologically relevant and holistic *in vitro* model of renal drug handling.
- Double transfected monolayer assays, on the other hand, provide *in vitro* tools to investigate the specific transporters that could contribute to the active elimination of either organic cationic (**Figure 2**: MDCKII-OCT2/MATE2-K and **Figure 3**: MDCKII-OCT2/MATE1) and anionic (**Figure 4:** MDCKII-OAT1/BCRP and **Figure 5:** MDCKII-OAT3/BCRP) compounds
- Altogether, double transfected cell lines with key renal transporters paired according to their overlapping substrate specificities, partnered with the PTC monolayers will give a powerful holistic insight into the impact of transporters on renal clearance.