Transporters Involved in the Renal Excretion of Chlorothiazide
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Purpose
The thiazide diuretic chlorothiazide exhibits low passive permeability and solubility, and is not metabolized, with the majority of the drug eliminated unchanged in the urine. The mechanism of chlorothiazide excretion remains poorly understood. We have recently demonstrated that chlorothiazide is actively taken up by the human organic anion transporter (OAT) proteins 1 and 3 (Juhász et al., 2013) localized at the basolateral membrane of renal proximal tubule cells (PTC). Furthermore chlorothiazide is a substrate of ATP-binding cassette transporter ABCG2 (BCRP/MXR) (Beery et al., 2012). These findings suggest that chlorothiazide is actively taken up into the proximal tubule cells and excreted by BCRP at the apical site for an efficient renal elimination. To support our hypothesis we have constructed a double-transfected Madin-Darby canine kidney (MDCK II) cell line permanently expressing OAT1 in the basolateral membrane and BCRP in the apical membrane.

Methods
Cellular accumulation studies were conducted on CHO and HEK cells transfected with OAT1 and OAT3, respectively. Vectorial transport was assessed on OAT1/BCRP double as well as single and parental control MDCKII cell monolayers.

Results
Chlorothiazide uptake by OAT1 was saturable, with a KM value of 14.5 µM, and Vmax of 108 pmol/mg protein/min. The basolateral-to-apical transport rate of chlorothiazide was seven times higher on OAT1/BCRP cell monolayers than either single transfected or parental background cell monolayers.

Conclusion
Our data suggest that OAT1, OAT3 and BCRP play a pivotal role in the vectorial transport of chlorothiazide across renal proximal tubule cells. The established OAT1/BCRP double transfected cell line is a promising model for active renal elimination of substrate drug molecules.