Supplementary Material

Endogenous plasma kynurenic acid in human: a newly discovered biomarker for drug-drug interactions involving in OAT1 and OAT3 inhibition

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Figure 1

The renal clearance (CLr) (A), renal secretion clearance (CLsec) (B) and renal extraction ratio (ERr) (C) of kynurenic acid in the subjects after oral administration of furosemide alone (phase 2) or coadministration of probenecid plus furosemide (phase 3). Fifty µL of urine samples were mixed with 200 µL ice-cold acetonitrile containing 200 nM of kynurenic acid-d5 as internal standard in 96-well plate. The samples were vortex-mixed and centrifuged at 4°C, 3700 rpm for 15 minutes. An aliquot of 50 µL of the supernatant from each well was transferred to a clean 96-well plate and diluted with 450 µL of 25% acetonitrile in water. An aliquot of 10 µL was injected to the LC-MS/MS system for analysis. The CLr, ERr and CLsec were estimated by the equations as follow:

$$\boldsymbol{CLr} = \frac{\boldsymbol{X_{0-24}}}{\boldsymbol{AUC_{0-24}}}$$

$$\boldsymbol{ERr} = \frac{\mathbf{CLr}}{\boldsymbol{f_u} * \mathbf{GFR}}$$

$$\boldsymbol{CLsec} = \mathbf{CLr} - \boldsymbol{f_u} * \boldsymbol{GFR}$$

where the $X_{0\text{-}24}$ is the cumulative amount excreted in the urine; $AUC_{0\text{-}24}$ is the area under the plasma concentration-time curve from 0 to 24 hrs; G*FR* is the glomerular filtration rate in human (125 mL/min) (Davies and Morris, 1993); f_u is the plasma unbound fraction of kynurenic acid (0.05) (van Gelder et al., 2020). The data are expressed as mean ± standard deviation (SD). Statistical differences between two group were determined using a paired two-tailed Student t-test in GraphPad Prism (version 7, GraphPad Software, Inc.; San Diego, CA). P<0.05: statistically significant.

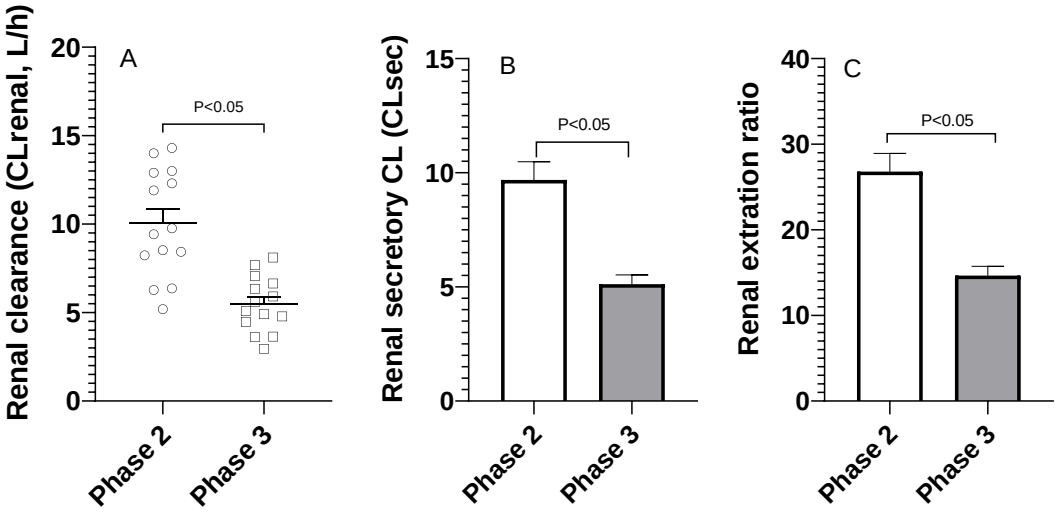
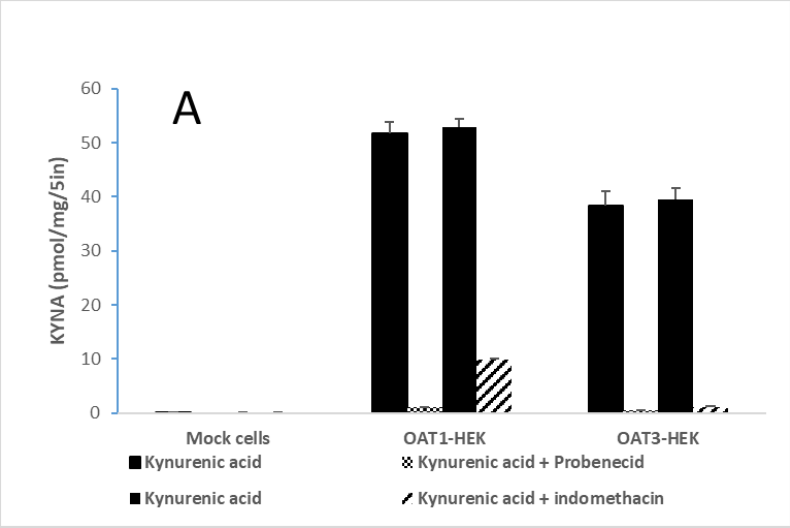
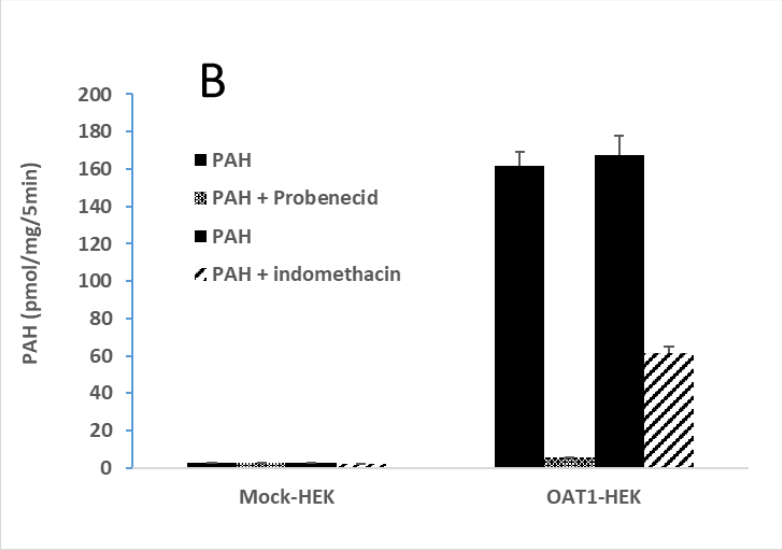
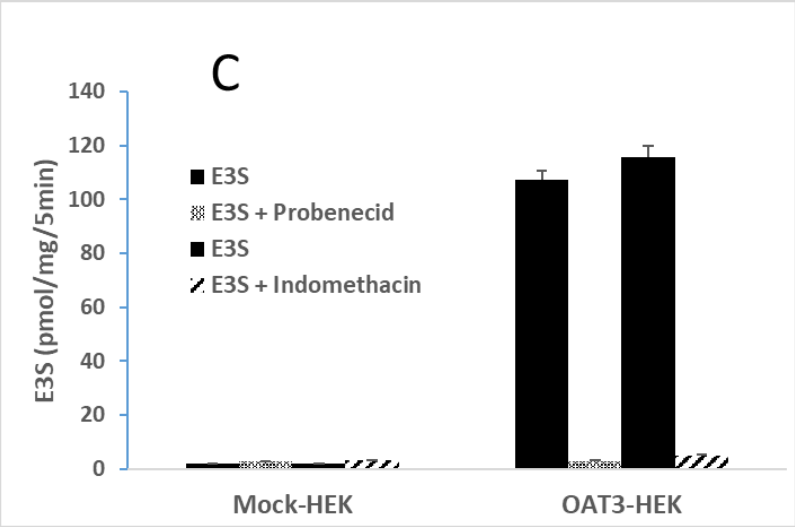


Figure 2. Measurement of kynurenic acid uptake in HEK cells lines constitutively expressing OAT1 or OAT3 transporter (OAT1-HEK and OAT3-HEK). The cellular uptake of kynurenic acid was tested at 0.5uM based on the physiological plasma concentrations and ensured analytical sensitivities. Probenecid (1mM) and indomethacin (100uM) was used as OAT inhibitors in the studies. The uptake of kynurenic acid in OAT1- and OAT3-HEK cells was approximately 727-and 541-fold higher than that in the control cells (51.6±2.26 and 38.4±2.57 vs 0.071±0.012 pmol/mg after 5 minutes, respectively) (A). In addition, the kynurenic acid uptake in OAT1- and OAT3-HEK cells is significantly inhibited by probenecid or indomethacin, OAT1 and OAT3 inhibitors (A). The positive controls, p-aminohippurate (PAH, 1 µM) (B) and extro-3-sulfate (E3S, 1 µM) (C) demonstrated OAT1 and OAT3 activities, respectively.







Reference

Davies B and Morris T (1993) Physiological parameters in laboratory animals and humans. *Pharm Res* **10:**1093-1095.

van Gelder MK, Middel IR, Vernooij RWM, Bots ML, Verhaar MC, Masereeuw R, Grooteman MP, Nube MJ, van den Dorpel MA, Blankestijn PJ, Rookmaaker MB, and Gerritsen KGF (2020) Protein-Bound Uremic Toxins in Hemodialysis Patients Relate to Residual Kidney Function, Are Not Influenced by Convective Transport, and Do Not Relate to Outcome. *Toxins (Basel)* **12**.