Identification of endogenous biomarkers of renal organic cation transporters in rats by global metabolomics analysis

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Kidney is one of the major drug-elimination organs. The total renal excretion of a compound is the net result of glomerular filtration, tubular secretion, and reabsorption. Tubular secretion is a transporter-mediated process, which is often mediated by organic cation transporters (OCT/Oct) that facilitate the active secretion of several cationic substrates including drugs such as metformin as well as endogenous cations. Endogenous biomarkers of OCTs can help in the early assessment of drug-drug interaction without the administration of exogenous OCT probe substrates. We hypothesize that administration of cimetidine, an OCT/Oct inhibitor, will lead to increased plasma levels and decreased renal clearance (CLR) of endogenous OCT/Oct substrates. Such substrates can potentially act as OCT/Oct biomarkers.

We carried out a rat pharmacokinetic (PK) study where metformin (5 mg/kg, IV) was administered as an exogenous substrate of OCT (positive control) to four Sprague-Dawley rats with and without cimetidine (100 mg/kg, IP) in a cross-over study design with one week washing period. Blood samples were collected from the tail vein at different time points, i.e., pre-dose, 0.17, 0.5, 1, 2, 4, 6, and 8 h, and urine samples were collected at 0-4 and 4-8 h intervals. Rat blood and urine samples were analyzed for metformin and cimetidine levels by a validated method using liquid chromatography with tandem mass spectrometry (LC-MS/MS) (Waters Xevo-TQ-XS MS; Waters, Milford, MA). Metformin area under the blood concentration-time curve (AUC0-8h) was significantly increased by 3.2 folds when co-administered with cimetidine (p-value, 0.003). Similarly, metformin CLR0-8h was significantly decreased in cimetidine arm by 3.7 folds (p-value, 0.029). Further, to investigate the effect of cimetidine on endogenous metabolites, we carried out untargeted metabolomics for rat blood and urine samples using Easy Spray 1200 series nanoLC coupled Q-Exactive HF MS (Thermo Fisher Scientific, Waltham, MA). The rat blood samples were analyzed in three groups, i.e., pooled (0.5, 1, and 2h), 0.5 h, and 1 h samples, while rat urine samples were analyzed at 0-4 h interval samples. The generated data were analyzed by open-access XCMS Online software (xcmsonline.scripps.edu). Greater than 18,000 features were detected in the blood which were shortlisted using optimized selection criteria, i.e., fold differences (versus without cimetidine) of 1.9-10 fold, p-value < 0.05, reproducible retention time, and quality of chromatogram peak. Out of the 85 shortlisted hits, 52 were detected by METLIN software, and 30 were common in blood and urine samples. Among several potential compounds predicted by METLIN for each mass-to-charge ratio (m/z) value, only compounds containing nitrogen atoms with mass error (ppm) less than 4 were selected. Two significant hits (m/z, 134.06, and 233.09 corresponding to putative oxindole and robustine, respectively) were consistently found in pooled, 0.5 h, and 1 h samples (Figure 1). Other putative metabolites were oxyquinoline (m/z, 146.09), deoxyadenosine (m/z, 252.11), 1-naphthylamine (m/z, 182.04), and 2-methylquinoline-3,4-diol (m/z, 176.07). A clinical study is currently being conducted to further validate these preliminary data.

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Figure 1: LCMS signal intensity with and without cimetidine of two significant hits found in the pooled, 0.5 h, and 1 h samples.