CORRELATING DIFFERENTIAL ENDOGENOUS METABOLITE PROFILES WITH THE PHARMACOKINETICS OF GEFITINIB AND ITS ASSOCIATED DRUG METABOLITES USING AN ION MOBILITY BASED APPROACH

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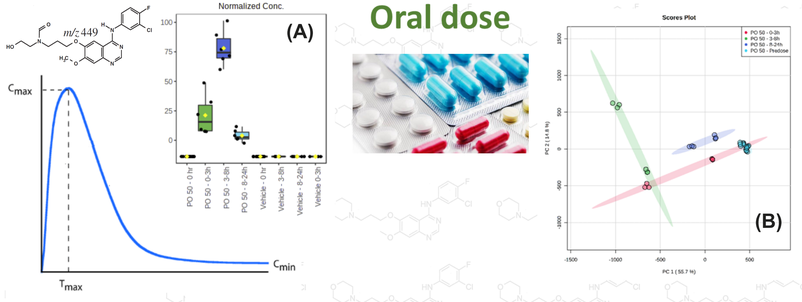
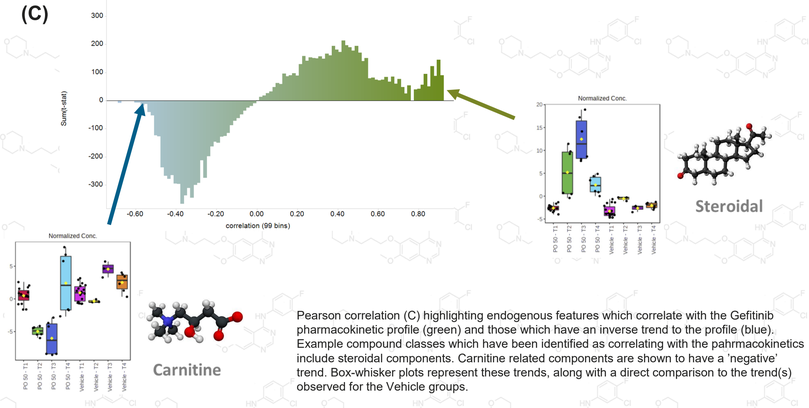
INTRODUCTION

Gefitinib (iressa), a selective inhibitor of the epidermal growth factor receptor (EGFR; HER1) tyrosine kinase, used for treating non-small cell lung cancer, is extensively metabolized in animals and humans. However, the methods used to determine its metabolic fate employed HPLC separations of 15-45 minutes duration, which are incompatible with modern high-throughput drug discovery. However, technologies such as UPLC, ion mobility (IM) spectrometry and high-resolution MS (HRMS), when combined, can achieve high-throughput without compromising quality. Thus, UPLC provides excellent separation efficiency allowing rapid analysis, IM offers an additional separation, resolving co-eluting interferences, and structurally relevant CCS values, whilst HRMS provides excellent data for metabolite identification. Here we applied UPLC-IMS-MS to characterize and identify Gefitinib-related metabolites and endogenous metabolite changes in the mouse urine.



Urine analysis using for the orally dosed group (PO) using the HILIC-IMS-MS methodology allowed for the identification of Gefitinib-related metabolites and endogenous species. An example drug metabolite originating from Gefitinib is at m/z 449, providing the characteristic pharmacokinetic profile. The box-whisker plot (A) shows the elevated intensity over time, reaching a C_{max} at 3-8 hrs before returning to comparative initial levels at 8-24 hrs.

Unsupervised PCA plots (pareto scaling applied) representing the PO group with Gefitinib and its related metabolites subtracted from the data (B). The trend observed for changes at the endogenous metabolite level reflects that of the expected pharmacokinetic profile as shown with (A) (i.e. deviation from the predose level before returning close to original, predose levels).



Pearson correlation (C) highlighting endogenous features which correlate with the Gefitinib pharmacokinetic profile (green) and those which have an inverse trend to the profile (blue). Example compound classes which have been identified as correlating with the pahrmacokinetics include steroidal components. Carnitine related components are shown to have a 'negative' trend. Box-whisker plots represent these trends, along with a direct comparison to the trend(s) observed for the Vehicle groups.

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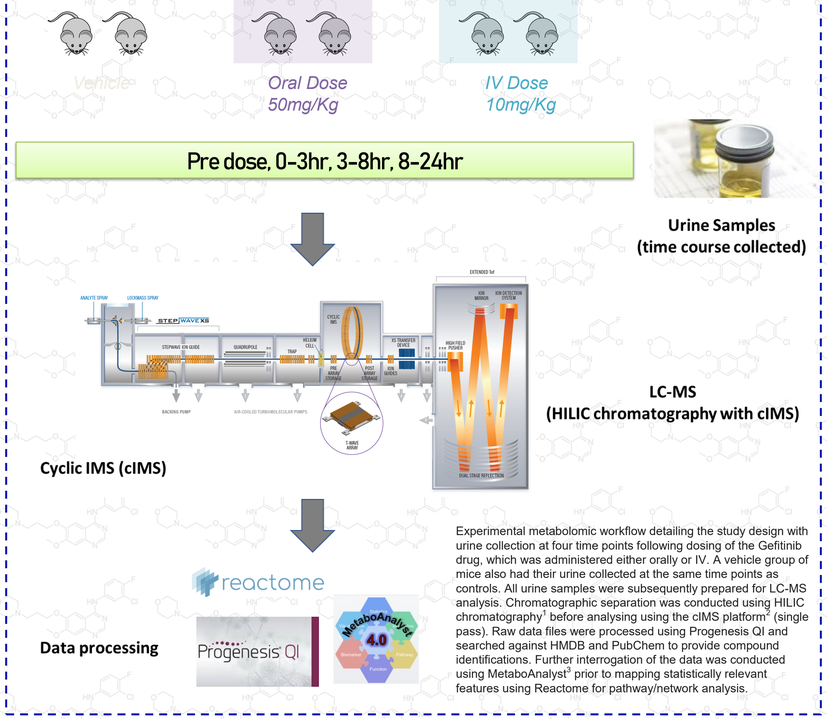


Experimental metabolomic workflow detailing the study design with urine collection at four time points following dosing of the Gefitinib drug, which was administered either orally or IV. A vehicle group of mice also had their urine collected at the same time points as controls. All urine samples were subsequently prepared for LC-MS analysis. Chromatographic separation was conducted using HILIC chromatography[1] before analysing using the cIMS platform[2] (single pass). Raw data files were processed using Progenesis QI and searched against HMDB and PubChem to provide compound identifications. Further interrogation of the data was conducted using MetaboAnalyst[3] prior to mapping statistically relevant features using Reactome for pathway/network analysis.

CONCLUSIONS

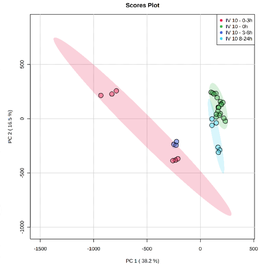
- **Utilising a HILIC-IMS-MS approach for polar metabolite analysis of urine has been applied and shown to provide comprehensive metabolomic insight over a 24 hr period, following administration of the Gefitinib drug.**
- **Identified drug metabolites demonstrated abundances at the respective time points, which correlated with known pharmacokinetic profiles. Additionally, the same data also allowed for endogenous metabolites not only to be identified but also provided the same pattern correlation as the drug metabolites.**
- **A variety of compound classes were identified as correlating metabolites, including carnitine, steroidal and dipeptide components.**
- **Biological significance of the identified endogenous metabolites were further evaluated using pathway tools, indicating changes within the spermidine/spermine biosynthesis, steroidgenesis and amino acid metabolism.**

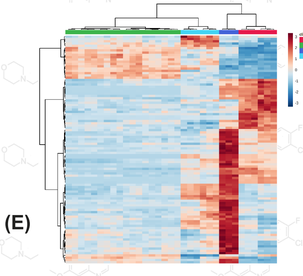
References

1. Spagou K et al. HILIC-UPLC-MS for Exploratory Urinary Metabolic Profiling in Toxicological Studies. Anal. Chem. **2011**, 83, 382-390.
2. Giles K, Ujma J, Wildgoose J, et al. A Cyclic Ion Mobility-Mass Spectrometry System. *Anal Chem.* 2019;91(13):8564-8573.
3. Chong, J et al. Using MetaboAnalyst 4.0 for Comprehensive and Integrative Metabolomics Data Analysis. **2019**, Current Protocols in Bioinformatics 68, e86

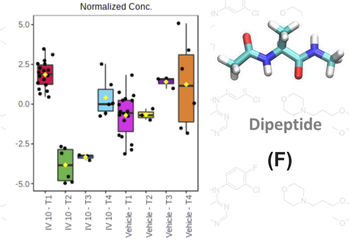
IV dose

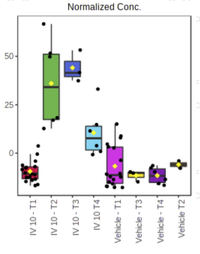
Data resulting from the IV administration of Gefitinib is provided. The resulting PCA plot (D) mirrors the PCA obtained for the oral dosed results (i.e. corresponding trend with the pharmacokinetic profile). Differential expression changes are also clearly visible using the top 100 statistically relevant features (based on t-test), represented here as a heatmap (E). Hierarchial clustering at the sample level shows individual mice (per group) and associated technical replicates to group accordingly.

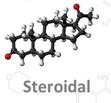


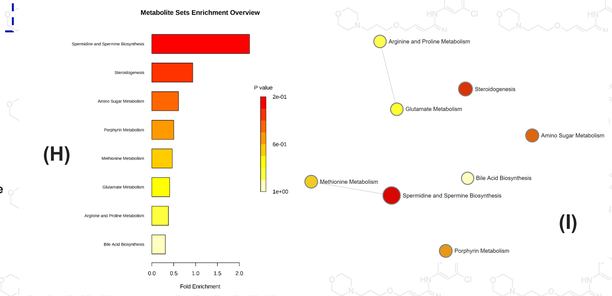


Based on the same approach as used for the oral dosed group, pattern searching was conducted for statistically relevant features using Pearson correlation in order to identify metabolites which showed positive/negative correlation with the pharmacokinetic profile. Two example classes of compound identified, included a range of dipeptides (negative correlation) and steroidals (positive correltaion). Corresponding box-whisker plots for both compound types are shown (F & G).









Statistically relevant features were mapped against pathways using enirchment tests. A number of biological processes were highlighted as being perturbed. Connections between certain processes were also identified. In particular, signal transduction, metabolism and the immune system were shown to be affected.

Individual pathways were ranked according to P-value with spermidine and spermine biosynthesis, steroidogenesis and amino sugar metabolism ranked as the top 3 significant pathways (H). Associations between these pathways were also identified as shown in figure (I).