Investigating The Relationship Between Off Target Pharmacology & The DMPK of Methapyrilene Using Lipidomics And MS Imaging. Abstract ID number: 322543

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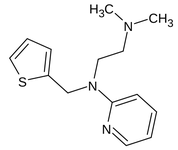
INTRODUCTION

Omics-based biomarker technologies including metabolomics and lipidomics are making a significant impact on disease understanding, drug development, and translational research. A wide range of pathophysiological processes involve lipids and metabolites therefore monitoring changes in their levels can give valuable insights into drug toxicity and off target pharmacology [1-3].



Methapyrilene.

Methapyrilene (Figure 1), an antihistamine and anticholinergic, developed in the 1950's for the treatment of insomnia was removed from the market in 1970 as it was demonstrated to cause cancer in rats following chronic administration and is a known hepatotoxin [4].

The aim of this study was to investigate the following:-

- Toxicokinetics & metabolic fate of methapyrilene following repeat dosing.
- Dysregulation of endogenous metabolites due to methapyrilene dosing.
- Spatial disposition of methapyrilene, and dysregulated metabolites & lipids in liver tissue.
- Identify dose and time dependent trends in lipid dysregulation as a result of drug exposure.

Ethics Statement

The study was performed at Evotec SAS (Toulouse, France) after full management review and in accordance with National and EU guidelines.

STUDY DESIGN

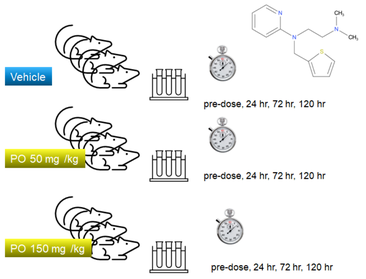
8 male Wistar rats were divided into 3 groups and were orally dosed with methapyrilene at either 0, 50 or 150 mg/kg/day for 5 days.

Sampling:-

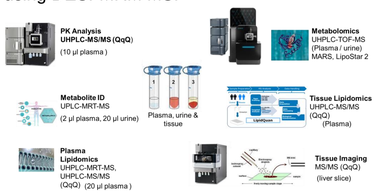
Urine, plasma, tissue and faeces samples were taken at 24, 72 or 120 h post dose from 2 animals per dose group.

Biofluid and tissue samples divided for :-

Toxicokinetics, metabolite ID, metabolomics, plasma, lipidomics, tissue imaging and clinical chemistry measurements.



Toxicokinetic analysis analysis was performed using reversed-phase UHPLC_MS/MS, metabolite identification, discovery metabolomics/lipidomics were performed using UHPLC-HRMS, discovery, quantitative lipidomics was performed using HILIC-MS/MS. Tissue imaging was carried out using DESI-MRM MS.



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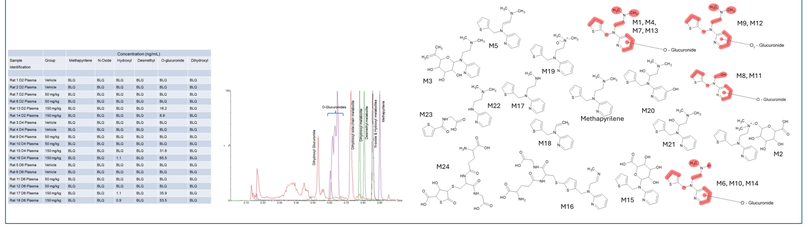
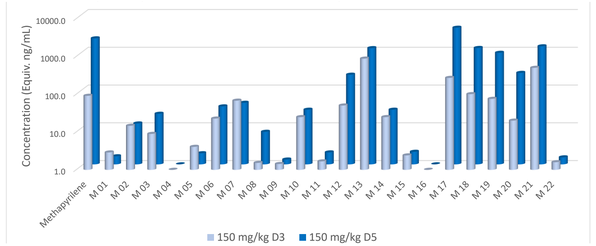
TOXICOKINETICS & BIOTRANSFORMATION'S

All of drug related material was eliminated from the plasma by the 24 h time point. Metabolite identification was performed using reversed – phase employing a 0.5 ppm mass filter. The data was analyzed using MassMetaSite, a total of 24 drug metabolites were identified including, loss of thiophene ring, demethylation, oxygenation, N—glucuronidation and O-glucuronidation.



URINARY METABOLITE ELIMINATION

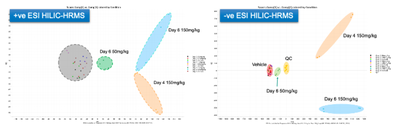
The urinary elimination of the drug metabolites was determined by reversed-phase UPLC-MS/MS in +ve ESI mode. There was a noticeable change in ratio of metabolite concentrations D3 – D5 for some metabolites. MP, M20, M8 increased whilst M1, M5 decreased.



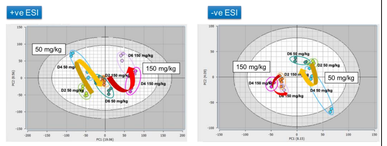
DISCOVERY METABOLOMICS & LIPIDOMICS

Discovery UHPLC-HRMS based lipidomics showed an observable temporal and dose trajectory in both in +ve and -ve ion mode. Different trajectories observed in the MVA for the 50 and 150 mg/kg dose groups. HILIC-HRMS metabolomics analysis of urine showed clear dose and temporal trajectories, with tight clustering of the QCs in all Studies.

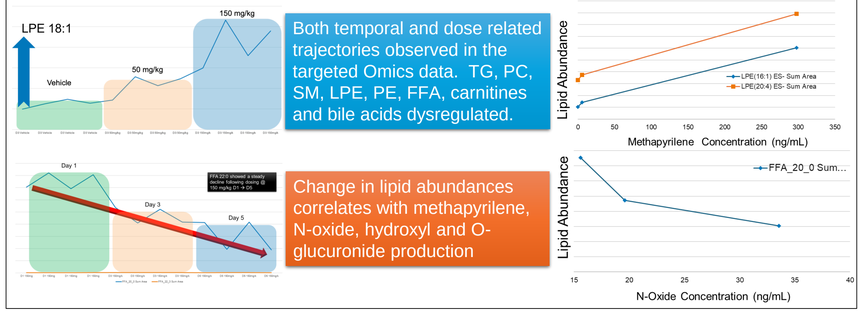
Urinary HILIC-HRMS Metabolomics



Plasma UHPLC-HRMS Lipidomics

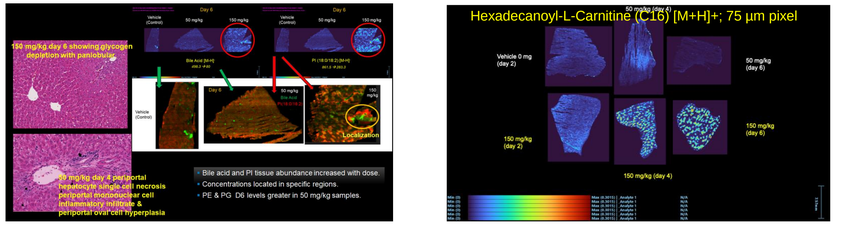


TARGETED LIPIDOMICS



TISSUE IMAGING

Liver tissues were analysed by DESI MS-QqQ imaging. It was noted that bile acid and PI tissue abundance increased with dose, whilst PE & PG D6 levels greater in 50 mg/kg samples. It can also be seen that dysregulated lipid concentrations were located in specific regions.



CONCLUSION

- **DMPK results were consistent with previous publications, several new glucuronide metabolites identified.**
- **Dysregulation of endogenous lipids following dosing showed both increases and decreases relative to control animals with carnitine, LPE, FFA, bile acids, LPE and PE profiles were the most disrupted by the administration.**
- **MS Tissue imaging of the liver tissue facilitated detection of methapyrilene and metabolites peak intensities occurred in D6 150 mg/kg samples.**
- **Bile acid and PI tissue abundance increased with dose, PE & PG D6 levels greater in 150 mg/kg samples. Lipid dysregulation was located in specific regions of the liver.**
- **Dysregulated endogenous metabolites correlated with urine metabolite elimination levels indicating pharmacolipidodymaic effects.**

References

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