A study of drug metabolism in zebrafish larvae using MALDI-MS Imaging

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Introduction
In this study we identified metabolites of a drug and the distribution of its metabolites in zebrafish larvae using LC-MS/MS and MALDI-MSI. Zebrafish (Danio rerio, abbr. ZF) larvae have been investigated as a promising vertebrate model to study drug metabolism (DM) due to their easy handling in high-throughput in vivo metabolite identification workflows. Mass spectrometry imaging (MSI) is a powerful technology allowing for visualization and analysis of the spatial distribution of molecules without labelling or staining. Matrix-assisted laser desorption/ionization (MALDI)-MSI has previously been proven to be suitable for the analysis of small molecules such as drugs and their metabolites in tissue samples.

New approach of ZF larvae to study in vivo DM
Can ZF larvae help to predict human drug metabolism?

Method
- Toxicity/DM study
  - Administration (via microinjection or medium)
  - Toxicity
  - Metabolism
- Microscopy (phenotyping control)
  - LC-HRMS/MS (male/female, 10-fold)
- Safety data
- Metabolite ID
- Preliminary information on the metabolism in human

Tissue Distribution (MSI)1,2
- Administration (via microinjection or medium)
- Embedding (gelatin solution)
- Cryosectioning (10 µm, 20X)
- Matrix application (2.5 µl)
- Mass spectrometry imaging (solaX MRMS, Bruker)

Data processing® for HRMS and MSI (MetaboScape, SCI-3 Labs, Biocompare)

- Exposure for 1 day
  - ZF control (no drug)
  - 3F_ADB_parent compound
  - 180.2177, 3F_ADB
  - 394.2126, 5F_ADB
  - 5F_ADB
  - 396.1919
- Exposure for 3 days
  - ZF control (no drug)
  - 3F_ADB_parent compound
  - 180.2177, 3F_ADB
  - 394.2126, 5F_ADB
  - 5F_ADB
  - 396.1919

Results
- Comparison of the detected metabolites following different administrations
- Comparability of the metabolites detected in three models
  - ZF larvae, HepaRG cells, Human urine
  - Visualization of the spatial distribution of the parent drug in ZF larvae after exposure through medium
  - Exposure for 1 day
    - Distribution into dorsal and ventral regions
  - Exposure for 3 days
    - Distribution into dorsal and ventral regions
- Total number of detected 7N-5F-ADB metabolites

Summary
- Metabolites were compared among three different models (i.e., ZF larvae, HepaRG cells, human urine)
- Microinjection in different organs was applied in ZF drug metabolism model
- The application of new drugs to MSI method in ZF larvae
- The distribution of 7N-5F-ADB and its metabolites was studied using MSI

Conclusions
- ZF larvae model is a promising tool to study human drug metabolism
- MSI can help to optimize compound administration in DM model of ZF larvae

On-going works
- Application of new drugs to MSI method in ZF larvae
- Optimization of MSI method in zebrafish larvae
- Data interpretation integrated by DM and MSI results