The Association for Mass Spectrometry: Applications to the Clinical Lab
#20c

SOLID-PHASE MICROEXTRACTION (SPME) IN KIDNEY EXAMINATION – LC-MS/MS BASED IDENTIFICATION OF POTENTIALLY SIGNIFICANT METABOLITES IN GRAFT QUALITY ASSESSMENT

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Introduction

Kidney transplantation is the treatment of choice for large number of people suffering from end-stage renal disease all across the world. Regrettably, even nowadays transplantology suffers from the lack of reliable methods of organ quality assessment. The standard protocols are limited to macroscopic appearance inspection or invasive tissue biopsy which do not provide a comprehensive information about the graft. Kidney is the organ largely associated with metabolic processes, thus measurements of metabolites concentrations may permit determining potential organ quality biomarkers and predicting the graft outcome. Hence, there is a need for new diagnostic solution allowing *on site* graft monitoring and quick decision-making processes during the surgery.

The goal of this project is to identify metabolites associated with changes occurring in transplanted kidneys during preservation with the use of *in vivo* and *in* situ low-invasive solid phase microextraction (SPME) followed by LC-MS/MS instrumental analysis.

Methods

- SPME probes coated with 7 mm mixed-mode (C18/benzenosulfonic acid) sorbent were directly inserted into the tissue for 30 min each time.
- The study was conducted on two types of porcine model donors: heart beating donor (HBD) and donor after cardiac death (DCD).

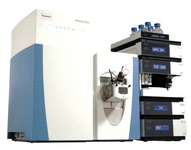
Extraction

- *in vivo* before transplantation, additionally for DCD after 45 min and 2h of warm ischemia time.
- *in situ* after 1h, 3h, 5h, 7h of perfusion,
- *in vivo* 3 and 7 days after revascularization in the recipient, and additionally for DCD after 45 min and 2h of warm ischemia time.

Desorption

- 250 µl ACN:H_2O (80:20; V/V)
- Desorption time = 60 min

LC-MS/MS analysis



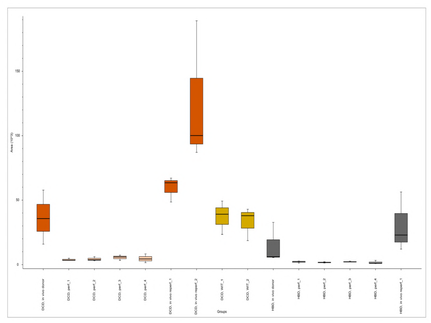
- Orbitrap Q-Exactive Focus
- ESI+
- RPLC: Supelco Discovery HS F5, 2.1 mm x 100 mm x 3µm
- A: H_2O /FA (99,9:0,1; v/v), B: ACN/FA (99,9:0,1; v/v)
- CID
- Collision energy: 10, 30, 60 eV

Data analysis

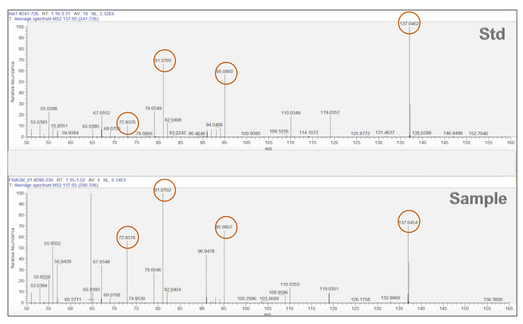
- Compound Discoverer 2.1
- QC-based area correction:
  - min 80% coverage,
  - max 30% RSD in QC
- normalization “constant mean”
- Fold Change ≧ 2
- P-value<0.05
- Database: KEGG, KEGG

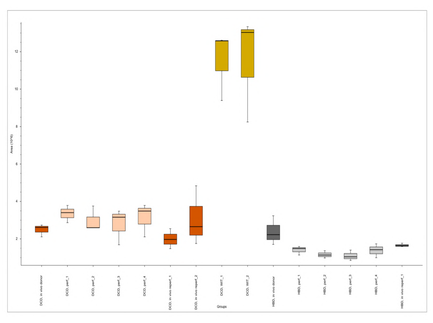
- The putative identification of metabolites detected in nontargeted analysis was done by comparison of accurate masses with metabolomic databases. In order to confirm identities of metabolites selected as significant ones in organ quality assessment, their retention times and fragmentation pattern were compared with chromatograms and MS/MS spectra of authentic standards.

Results and discussion

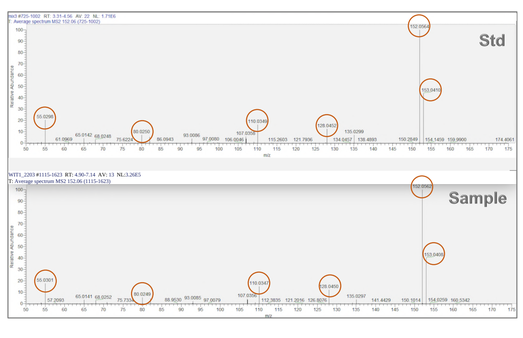


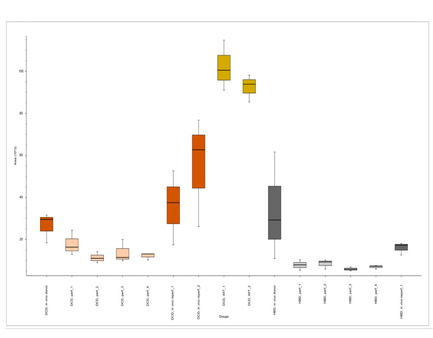
Gamma-aminobutyric acid
- Induces a significant protective effect against oxidative injury.
- Ameliorates kidney functions induced by renal ischemia-reperfusion injury.



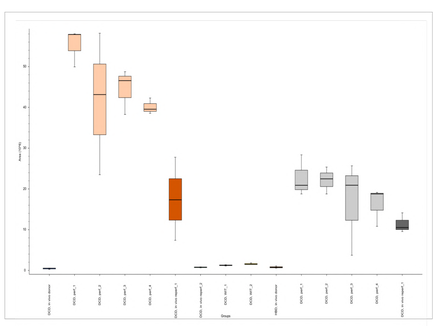


Hypoxanthine
- Warm ischemia time leads to hypoxanthine accumulation in the graft.
- One of the most important factors participating in enzymatic ROS generation within ischemic tissues.
- It has been previously reported that hypoxanthine levels during WIT in HBD correlates with graft viability in the recipient.

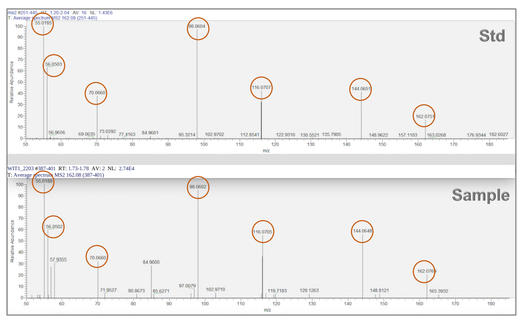


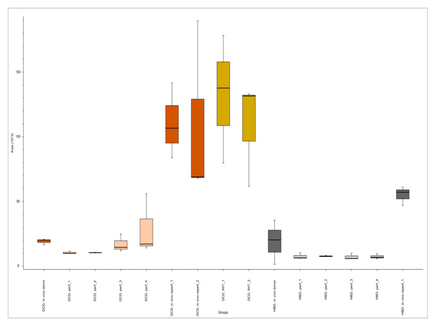


Guanine
- Significantly decreases during perfusion and increases in warm ischemia time compared to *in vivo* donor and recipient.
- One of the five main nucleobases found in the nucleic acids in DNA and RNA.

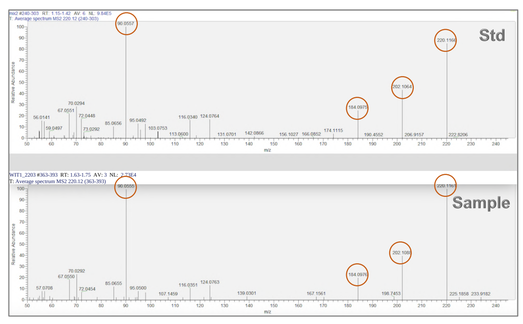


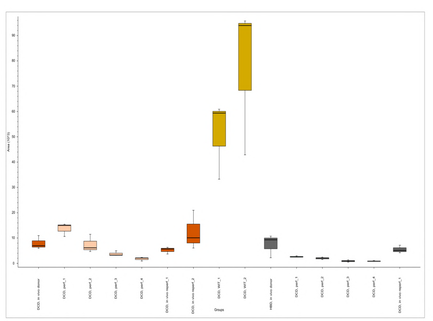
Histidine
- Is an essential alpha-amino acid, a precursor of histamine biosynthesis.
- Alterations of histidine levels are concerned to be related with protein-energy wasting, systemic inflammatory processes, oxidative stress and a higher risk of mortality in patients with chronic kidney disease.
- Significant increase during preservation.





2-aminoadipic acid
- Is a potential small-molecule marker of oxidative stress.
- Increased levels of 2-aminoadipic acid were noticed in patients with acute and chronic renal failure.





Pantothenic acid
- Plays a protective role in ischemia-reperfusion-induced renal injury.
- Takes part in the disposal of ROS by increasing glutathione and the repair of cell membranes and tissue injury by increasing CoA and ATP synthesis.

Monitoring the metabolomic profiles of kidneys allowed to observe biochemical changes occurring in the organ during preservation as well as biochemical differences between HBD and DCD types of donor. Alterations are mostly related to concentrations of metabolites which might be the part of ischemia/reperfusion injury mechanism due to their involvement in i.e. oxidative stress and ischemia pathways.

Conclusions

- SPME is a low-invasive method for direct *in vivo* kidney extraction without removing any tissue from the graft which makes the method an alternative to biopsy.
- The small size probe and minimal invasiveness of the approach permits for repeatable samplings from the same organ.
- Identified metabolites could be considered for further analyses towards decreased organ quality or progressing graft dysfunction biomarker validation.

References

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- B. Bojko et al., “Low invasive in vivo tissue sampling for monitoring biomarkers and drugs during surgery,” Lab. Investig., vol. 94, no. 5, pp. 586–594, 2014.

Acknowledgments

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