THESIS OFFER (3.5 years)

OMICs workflow for Acute Kidney Injury (OWAKI)

Keywords : Fluxomics, Metabolomic, High resolution mass spectrometry, Acute Kidney Injury

Description:

Acute Kidney Injury (AKI) is a devastating condition occurring in one out of two patients admitted in an Intensive Care Unit (ICU). AKI is associated with poor outcomes including transition to chronic kidney disease and mortality. Despite several trials, no treatment has been shown effective and many aspects of AKI pathophysiology remain unknown. Renal gluconeogenesis, the reverse pathway of glycolysis, is decreased during AKI in association with mortality (Verissimo et al., 2022).

In order to understand the mechanisms at play during AKI, we propose to set up analytical and computer tools capable of identifying the metabolic pathways active during the occurrence of AKI.

To do this, we will then assess the energetic metabolism through ^{13}C isotopic tracers resolved metabolomics. Uniformly labeled palmitate, glucose and lactate will be delivered to mice before sacrifice as a single bolus intravenous injection. Labeled substrate will be thus metabolized via fatty acid oxidation, glycolysis and gluconeogenesis respectively, producing specific labeled products that will further be identified in the harvested kidney tissues. ^{13}C-enrichment ratio of intermediate metabolites from the glycolysis/gluconeogenesis, Pentose Phosphate Pathway (PPP), Fatty Acid beta-Oxidation (FAO) and Tricarboxylic Acid (TCA) cycle will be measured and normalized by the ^{13}C-enrichment ratio of the initial substrate to alleviate potent change in substrate delivery rate.

Based on previously methodological approaches developed by our laboratory (Acket et al., 2015, 2017, 2020) and recent advances on profiling metabolic changes (Liu et al., 2019), the metabolite enrichment of a substantial number of intermediates of the aforementioned pathways will be analyzed by LC-Hilic-ESI-QTOF. The implementation of this analytical method will make possible the study of more than one hundred polar metabolites by both Mass Isotopomer Distribution Analysis (MIDA) and Principal Component Analysis (PCA). The main objective will be to set up and validate an analytical method and to carry out the analyzes to identify, by non-targeted metabolomics approach, the group of metabolites that significantly change their concentration under the different experimental conditions. Moreover, in order to analyze the central carbon metabolic network through the implementation of Flux Balance Analysis (FBA) (Degournay, 2018), the previous analysis will be completed with the quantification of key non-enriched metabolites.

The general goal consists in recognizing the most active metabolic pathways associated to AKI, particular attention will be given to glycolysis, gluconeogenesis and beta-oxidation. In light of the information gathered, quantification of isotopic enrichment in cellular ^{13}C-labeled metabolites (MIDA) from the accurate measurement of their isotopic mass by High Resolution Mass Spectrometry (HRMS) will allow the identification of the most active metabolic routes related to AKI. In particular, flux analysis through Mass Isotopomer Multi-Ordinate Spectral Analysis (MIMOSA) will be used for detecting changes in central carbon metabolism (Alves et al., 2015).

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Methodologies/Techniques

The project revolves around an analytical development on the techniques of chromatography and high resolution mass spectrometry. The data will be reprocessed using proprietary tools (Agilent Mass profiler Pro, Agilent Mass hunter, Matlab) and free tools such as R or XCMS. The animal experiments will be carried out by the partners associated with the project (CHU de GENEVE).

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