Journal of Pharmaceutical and Biomedical Analysis 161 (2018) 313–325

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Review

Metabolomics in chronic kidney disease: Strategies for extended metabolome coverage

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ARTICLE INFO

Article history:
Received 12 June 2018
Received in revised form 22 August 2018
Accepted 23 August 2018
Available online 25 August 2018

Keywords:
Chronic kidney disease
Metabolomics
Analytical coverage
Multi-platform
Multi-blocks

ABSTRACT

Chronic kidney disease (CKD) is becoming a major public health issue as prevalence is increasing worldwide. It also represents a major challenge for the identification of new early biomarkers, understanding of biochemical mechanisms, patient monitoring and prognosis. Each metabolite contained in a biofluid or tissue may play a role as a signal or as a driver in the development or progression of the pathology. Therefore, metabolomics is a highly valuable approach in this clinical context. It aims to provide a representative picture of a biological system, making exhaustive metabolite coverage crucial. Two aspects can be considered: analytical and biological coverage. From an analytical point of view, monitoring all metabolites within one run is currently impossible. Multiple analytical techniques providing orthogonal information should be carried out in parallel for coverage improvement. The biological aspect of metabolome coverage can be enhanced by using multiple biofluids or tissues for in-depth biological investigation, as the analysis of a single sample type is generally insufficient for whole organism extrapolation. Hence, recording of signals from multiple sample types and different analytical platforms generates massive and complex datasets so that chemometric tools, including data fusion approaches and multi-block analysis, are key tools for extracting biological information and for discovery of relevant biomarkers. This review presents the recent developments in the field of metabolomic analysis, from sampling and analytical strategies to chemometric tools, dedicated to the generation and handling of multiple complementary metabolomic datasets enabling extended metabolite coverage to improve our biological knowledge of CKD.

Contents

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https://doi.org/10.1016/j.jpba.2018.08.046
0731-7085/

1. Introduction

Metabolomics is a potent approach for assessing phenotype modifications caused by pathologies or environmental influences at the molecular level and is based on the comprehensive monitoring of metabolites (mass <1000 Da) in biological systems. During last decades, trends in human clinical metabolomics focused not only on metabolite and biomarker discovery but also on understanding the interactions between the metabolites in metabolic pathways to infer network mechanisms and obtain biological information. Enrichment analysis allows to study the link between metabolites concentration and biological pathways: if the concentration of metabolites from a biological pathway is modified as a consequence of a disease or experimental factor, this pathway is expected to be involved and conversely. In this context, one of the major challenges of metabolic profiling is related to the coverage and reliability of the measured biochemical information. Metabolomic study requires extended coverage of metabolites to provide (i) the overall view of the metabolic alterations caused by a specific situation (*e.g.,* a pathology), (ii) a relevant assessment of biochemical phenotypes and (iii) a reliable evaluation of biological hypotheses. Two major approaches are commonly used: targeted and untargeted metabolomics [1,2]. Targeted metabolomics focuses on a defined set of metabolites driven by biological hypothesis, specific compound classes or metabolic pathways. As chemical properties are known for these metabolites, sample preparation and analytical conditions can be adapted. Therefore, metabolite identification and quantification are facilitated. In contrast, untargeted metabolomics aims to cover all the metabolites of the sample without any prior biological knowledge. With this approach, multiple examination of one dataset is possible depending on the biological information of interest. Metabolite annotation and identification are therefore the major bottlenecks of untargeted metabolomics. Strategies presented in this review focus mainly on untargeted approach as it is more relevant in terms of extended metabolome coverage but most of them can also be applied in targeted or multitargeted metabolomics.

A careful study design, biofluid type and analytical platform selection is necessary to analyze the links between the metabolites and biological alterations. All steps of sample storage and handling must be clearly monitored to prevent confounding factors or biases related to sample degradation [2–9]. Because the analysis of a single sample type is generally not sufficient for the whole organism extrapolation, multiple biofluids or tissues are often required. From an analytical point of view, it is currently not possible to monitor all metabolites within one run; therefore, multiple analytical techniques providing orthogonal information should be used in parallel for coverage improvement. Coverage improvement strategy can be implemented at every step of the metabolomics workflow: from sampling, to sample preparation and analysis. Recording of the signals from multiple sample types and different analytical platforms generates massive and complex datasets. Chemometric tools, including data fusion approaches and multi-block analysis, constitute key tools for the extraction of biological information and discovery of relevant biomarkers. Data integration with other "omics" sciences is also helpful for data interpretation and biological reliability [10–12].

Chronic kidney disease (CKD) is increasing worldwide and is becoming a major public health issue [13,14]. CKD is characterized by the progressive loss of kidney function leading to end-stage renal disease [15] with high risk of cardiovascular morbidity and mortality [16]. CKD is classified into five stages according to the estimated glomerular filtration rate (eGFR) calculated from serum creatinine, or the presence of albumin in the urine [17]. Stages 1 (eGFR >90 ml/min/1.73m^2) and 2 (eGFR = 89–60 ml/min/1.73m^2) are generally asymptomatic. From the stage 3 of the disease, characterized by a decline in glomerular filtration lower than 60 ml/min/1.73m^2, there is an increased prevalence of metabolic complications like acid-base balance or electrolytes disorders [18,19]. Dialysis or transplantation are mandatory at the stage 5 (eGFR <15 ml/min/1.73m^2) due to inability of kidneys to maintain homeostasis and keep healthy concentration of metabolites in blood and urine [18]. From early stages, alterations of metabolites concentrations can be observed in patients' urine and blood. Some toxic compounds are not eliminated by the kidney anymore and accumulate in blood. Other are produced by the diseased tubular cells and can be found in the urine. For several reasons, metabolomics is a good candidate for obtaining new insights into CKD and its numerous challenges: (i) CKD is generally asymptomatic at early stages and difficult to diagnose. Patient stratification remains therefore critical at early stages and new biomarkers are urgently needed for CKD prevention. Indeed, serum creatinine or cystatine C, and proteinuria are not accurate markers of renal function and can be normal even in the presence of renal disease. In addition, they can be affected by non-renal factors, such as age, nutrition or volemia. Kidney biopsy is an invasive tool which is performed only after careful evaluation in order to determine the cause of renal disease when a therapeutic strategy could be applied [20,21]. In that context, recent study showed the interest of using metabolomic profile to improve eGFR accuracy [22]. (ii) Biochemical mechanisms of kidney disease are complex and remain mostly unknown. An improved understanding of the disease will help the development of new therapeutic targets preventing or reducing kidney degradation. (iii) Proper monitoring of both hemodialysis and (iv) transplantation is needed to guarantee healthy metabolite blood concentrations for end-stage patients and also for donor individuals. Furthermore, metabolites already linked to the effects of CKD or hemodialysis are characterized by diverse chemical structures and physicochemical properties including both very polar molecules such as amino acids or sugars, and very apolar compounds such as lipids [23]. To date, only few metabolomics studies dedicated to kidney disease took advantage of multiple analytical platforms [24–26] or multiple biological compartments [27–29]. However, this type of approaches combined with adapted chemometric tools can help to provide an extended coverage of complex biofluids composed of thousands of metabolites and possibly provide new biological insights into the metabolic consequences of CKD and hemodialysis. This review describes the recent developments in the field, from sampling and analytical strategies to chemometric tools, dedicated to produce and handle multiple complementary metabolomic datasets leading to extended metabolite coverage in order to improve our biological knowledge of CKD.

2. Analytical aspects

Mass Spectrometry (MS) and Nuclear Magnetic Resonance (NMR) are the most popular platforms for metabolomic studies. The selectivity and sensitivity of modern high-resolution mass