Proteomics and Metabolomics as Tools to Unravel Novel Culprits and Mechanisms of Uremic Toxicity: Instrument or Hype?

William Mullen, PhD,[*] Daisuke Saigusa, PhD,[†] Takaaki Abe, MD, PhD,[‡,§,‖] Jerzy Adamski, PhD,[¶,#,**] and Harald Mischak, PhD, MD[*,††]

Summary: The development of proteomic and metabolomic technologies holds the promise to significantly impact patient management by improving diagnosis, unraveling more appropriate therapeutic targets, and enabling more precise prognosis of disease development. Proteomics and metabolomics have been applied with the aim of improving dialysis, defining uremic toxins, and unraveling their origin. Ideally, these technologies should inform us which proteomic or metabolomic compounds are subject to significant alterations of concentration or structure as a result of failing kidney function, and thus can be considered as potential uremic toxins. After a few years of applying these technologies in the area of uremic toxicity studies we are now in a position where we can estimate how and what they can contribute to the field. In this review we critically examine the current literature on the application of proteomics and metabolomics in the context of dialysis and uremic toxins. We highlight the most promising findings, indicate where we see the current need, and which future developments consequently are to be expected, given the technological constraints that undoubtedly exist.

Keywords: Proteome, metabolome, uremic toxin, dialysis, biomarker

Uremic toxins are a group of poorly defined molecules that are eliminated in healthy individuals via the kidney, and that accumulate in patients with end-stage renal disease (ESRD). Several molecules have been described as uremic toxins, for more details see the recent review by Duranton et al.[1] The different classes of uremic toxins and their representatives also are discussed in more detail in articles 1 through 5 of this issue.[2–6] However, it is unknown how comprehensive the list of uremic solutes summarized by Duranton et al[1] in fact is, and for many of them the presumed toxic effects in vivo are extensively evaluated and described. There is hope that both questions may be answered by generating an exhaustive list of compounds found in plasma of healthy individuals, and in patients with late-stage chronic kidney disease or ESRD patients. The compounds that differ between these two populations constitute the pool of potential candidate uremic toxins. The observed associations of several of these compounds with specific pathophysiology (eg, cardiovascular complications) is the first step toward defining their toxicity. With these goals in mind, it is obvious that samples must be evaluated (ideally plasma) that are collected from patients and controls (see later), to obtain information on the compounds involved, in a hypothesis-free approach. As such, proteomics and metabolomics have been applied in this context. After about 10 years of research, it is time to reflect on the results and to re-evaluate the findings and the strategies used.

METABOLOMIC TOOLS

The recent growth of metabolomics has depended greatly on nuclear magnetic resonance (NMR) spectroscopy (mostly 1H-NMR)[7] and the development of mass spectrometry (MS).[8] In general, MS, particularly liquid chromatography (LC), coupled online via electrospray ionization (ESI) to high-resolution Fourier transform ion cyclotron resonance MS, and NMR spectroscopy (mostly 1H-NMR) are the two major spectroscopic techniques used in metabolic analysis (Fig. 1). They both have specific advantages and disadvantages,[9,10] as also described later.

[*]*British Heart Foundation Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, United Kingdom.*
[†]*Department of Integrative Genomics, Tohoku Medical Megabank Organization, Tohoku University, Sendai, Japan.*
[‡]*Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan.*
[§]*Division of Medical Science, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan.*
[‖]*Department of Clinical Biology and Hormonal Regulation, Tohoku University Graduate School of Medicine, Sendai, Japan.*
[¶]*Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Experimental Genetics, Genome Analysis Center, Neuherberg, Germany.*
[#]*German Center for Diabetes Research, Neuherberg, Germany.*
[**]*Lehrstuhl für Experimentelle Genetik, Technische Universität München, Freising-Weihenstephan, Germany.*
[††]*Mosaiques Diagnostics, GmbH, Hannover, Germany.*

Financial support: Supported in part by EU funding through SysKID (HEALTH-F2-2009-241544) and grant GA 251368 (Protoclin) from the FP7-PEOPLE-2009-IAPP program.

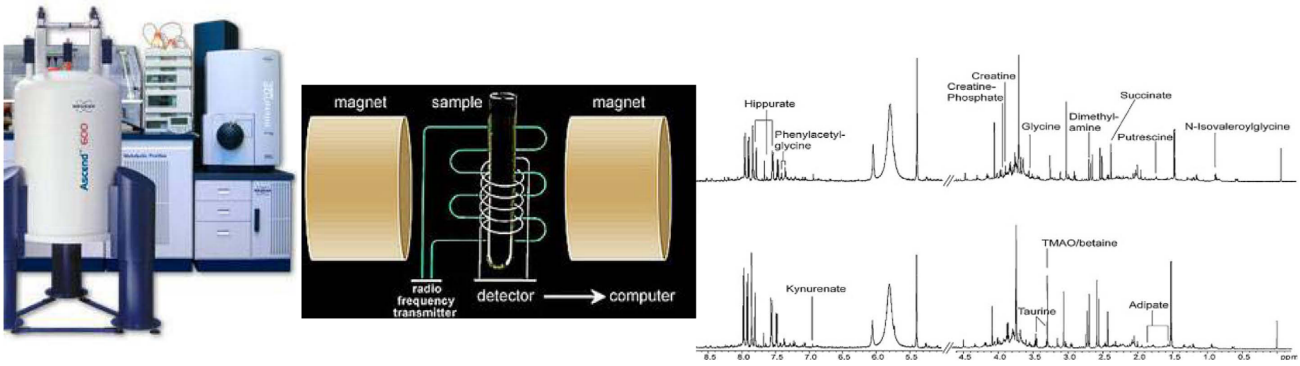
Conflict of interest statements: Harald Mischak is the founder and co-owner of Mosaiques Diagnostics.

Address reprint requests to William Mullen, PhD, British Heart Foundation, Glasgow Cardiovascular Research Centre, University of Glasgow, Glasgow, United Kingdom. E-mail: william.mullen@glasgow.ac.uk

0270-9295/ - see front matter

http://dx.doi.org/10.1016/j.semnephrol.2014.02.009

A



B

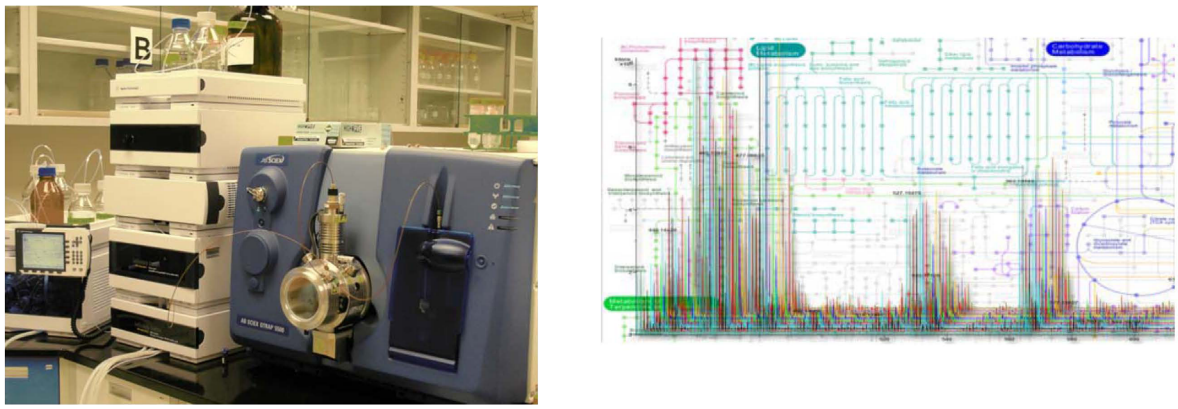


Figure 1. Commonly used metabolomic technologies. (A) Proton nuclear magnetic resonance (1H-NMR) is based on determining the resonance frequency in a strong magnetic field. This approach is of moderate sensitivity and resolution, but of low cost. (B) LC-MS–based approaches are of much higher sensitivity and resolution, however, also at a much higher cost.

MS determines the composition of molecules based on the mass-to-charge ratio in charged particles. MS-based metabolomics generally combines a first rapid global screening of untargeted metabolomics for searching candidate biomarkers using high-resolution MS, and, subsequently, a second determination screening of targeted metabolomics using tandem mass spectrometry (MS/MS). The advantages of MS (or MS/MS, these two instruments will to some degree be used synonymously in this article) are a wide dynamic range of detection, excellent sensitivity and selectivity, high throughput, reproducibility, and, depending on the instrument, high resolution. MS or MS/MS typically are interfaced with different separating devices, generally using ESI. Although ESI ideally is suited for polar charged molecules, nonpolar molecules may require chemical ionization. Several reports have been published using gas chromatography–mass spectrometry (GC/MS),[11] liquid chromatography–mass spectrometry,[12] and capillary electrophoresis–mass spectrometry (CE-MS)[13] for both untargeted and targeted metabolomics. In particular, time-of-flight and Fourier transform ion cyclotron resonance MS are useful for untargeted metabolomics,[14] and tandem quadruple MS is suitable for targeted metabolomics.[15] LC/MS is highly sensitive, typically at the high attomol level, and permits highly specific multiple metabolite assessments at low concentrations.[16] However, MS sensitivity is dependent on metabolite pKa and hydrophobicity,[17] and a widely adopted and validated methodology for sensitive, high-throughput discovery-based LC/MS metabolomics is still lacking. In part because of the heterogeneity in methods, the results from different groups using different experimental approaches are very divergent.

Furthermore, sample storage conditions and methods of extraction can affect and modify metabolite structure, confounding already complex data sets and introducing substantial additional variability. Despite the extensive use of MS to assess small molecules, a widely adopted and validated methodology for sensitive, high-throughput discovery-based LC/MS metabolomics is still lacking, and most compounds detected in MS-based metabolomics approaches are unknown/unidentified. Nevertheless, discovery metabolomics showed a wealth of possibilities in pharmaceutical and biomedical research.[18] To date, LC/MS–based