Metabolomic Profiling of Uremia With an LC-EC Array-MS Parallel Platform.

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Overview
Purpose: The objective of this project was to characterize the metabolomic profile of patients with uremia using a novel HPLC-electrochemical (EC) array—mass spectrometry (MS) platform.

Methods: Plasma samples were obtained from 28 uremic hemodialysis patients and 30 healthy control subjects. Gradient-coulometric array methods were developed for the global measurement of water-soluble analytes and for the targeted measurement of fat-soluble vitamins and antioxidants. Our novel parallel HPLC-EC-MS metabolomics platform was used to simultaneously profile multiple known and unknown redox and/or ionizable soluble compounds in plasma. EC and MS data were subjected to principal component analyses (PCA), and results from uremic samples were compared to samples from healthy subjects.

Results: In all cases, samples were readily differentiated from healthy controls by PCA. To our knowledge, this is the first metabolomics study specifically designed to assess the dysmetabolism that accompanies uremia. We have provisionally identified numerous redox active and/or ionizable metabolites whose concentrations are altered in this disease.

Introduction
Metabolites are key components of diverse biochemical processes, and changes in their concentrations can be related to the response of biological systems to environmental changes. The comprehensive metabolomic profile, i.e., the ‘metabolome’, can be viewed as the final stage in a chain of events ranging from gene function to metabolism, and thus the metabolite phenotype may be the most direct reflection of the current state of a biologic system.

Kidney function plays a critical role in maintaining circulating and organ system functional homeostasis, and loss of kidney function leads to dysregulation of many metabolic pathways leading to uremic syndrome. Since alterations in gene and protein expression are subject to complex homeostatic control and feedback mechanisms, it has been suggested that metabolomics may provide a higher level of integrated information than other ‘-omics’ approaches and be reflective of underlying pathophysiology. Thus, uremia may be an ideal disease state applicable to metabolomic studies.

Increased oxidative stress is a metabolic alteration known to contribute to the pathogenesis of alterations in renal function. While several authors have now identified increased oxidative stress state to date. Recent oxidative stress in uremia has primarily been evaluated by targeted analysis of the plasma concentration of individual oxidized solutes or antioxidants. Several studies have demonstrated an association between the plasma concentrations of individual oxidized solutes or antioxidants and subsequent mortality. However, there is a lack of a comprehensive assessment of uremia that incorporates simultaneous determination of multiple redox active and/or ionizable metabolites. Our novel platform allows the detection of these compounds.

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Methods

Study Subjects
Plasma samples were obtained from 10 patients with end-stage renal disease (ESRD) requiring hemodialysis therapy (shown immediately pre-dialysis) and from 10 healthy control subjects. The ESRD group was comprised of all Caucasian subjects (60% age 65-74 years). Since diabetes mellitus is the leading cause of ESRD, and diabetes may independently affect the circulating metabolite profile, we chose to study ESRD subjects with 0 ESRD subjects without diabetes mellitus. The healthy control group was comprised of all Caucasian subjects (5 men who were 61±7 years of age).

All subjects were between the ages of 18 and 85. No subjects had evidence of active malignancy, severe gastrointestinal dysfunction, or other serious clinical condition. Similarly, no subjects were taking vitamin E supplements (50 IU/day or less) or had a history of smoking or alcohol consumption. The Institutional Review Board approved the study. Patients were informed about the study, and written consent was obtained from all subjects.

Sample Preparation
Venous blood was drawn into Vacutainer® (Becton Dickinson, Franklin Lakes, NJ) tubes containing natrium heparin (1:100 v/v). Blood samples were centrifuged at 15,000 rpm for 10 min at 4°C. Plasma was removed and frozen within one hour of sample collection and stored at -70 oC until analysis.

Liquid Chromatography
The LC-EC-MS platform consisted of an HPLC system, and a linear ion trap mass spectrometer with an electrospinning condenser (ESI) source in parallel with a sixteen channel EC detector (Thermo Scientific Dionex CoulexArray Detector with Thermal Organizer).

Method 1: Water Soluble Metabolites
An LC method optimized for routine analysis of water soluble metabolites was used with EC detection. Mobile phase A consisted of 0.1 M monobasic sodium phosphate, 10 mg/mL sodium azide, and 50 mM potassium phosphate (pH 3.25). Mobile phase B consisted of 0.1 M monobasic sodium phosphate, 50 mg/mL SDS, 50 mM HEPES, 50% v/v, B, 0.1% v/v Triton X-100. A linear gradient was prepared by mixing 0% Mobile phase A and 100% B for 6 min, followed by a linear gradient to 40% B in 10 min, and a linear gradient to 90% B in 10 min, before returning to initial conditions. A C18 MD-150 column (150 mm x 3 mm, 3 µm) was held at 50°C, with a flow rate of 0.8 mL/min, and column temperature of 37°C. EC array potentials were held at -1100 mV (ESI+) and +1100 mV (ESI-). Data acquisition was performed with a Waters Xevo TQD mass spectrometer. Data were acquired as a centroid MS full-scan mode.

Method 2: Lipid Soluble Compounds
A novel method for profiling redox-active metabolites was used with EC detection alone. Mobile phase A consisted of methanol:0.2 M ammonium acetate (1:1, pH 4.4). Mobile phase B consisted of methanol:1 M ammonium acetate (70:20, v/v, pH 4.4). The gradient timeline was a linear gradient from 0%-60% B in 10 min, followed by a linear gradient to 100% B in 15 min, and then held at 100% B for 15 min, before returning to initial conditions. A C18 MD-150 column (150 mm x 3 mm, 3 µm) was held at 50°C, with a flow rate of 0.8 mL/min, and column temperature of 37°C. EC array potentials were held at -200 mV (ESI+) and +400 mV (ESI-). Data acquisition was performed with a Waters Xevo TQD mass spectrometer. Data were acquired as a centroid MS full-scan mode.

Method 3: Parallel LC-EC-MS Conditions
An LC method developed for the separation of a diverse range of analytes (i.e., metabolites soluble in aqueous and organic solvents) was used with parallel EC-array and MS detection. Mobile phase A consisted of 75% aqueous methanol (v/v) and mobile phase B consisted of 100% aqueous ACN (v/v). The gradient timeline was a linear gradient from 0%-60% Mobile phase B was used with a Waters Xevo TQD column (150 mm x 7.5 mm, 3.5 µm Xbridge BeLi Co Ltd). Data acquisition was performed with a Waters Xevo TQD mass spectrometer. Data were acquired as a centroid MS full-scan mode.

Conclusion
The coupling of MS in parallel with the EC array is a powerful analytical approach providing additional, orthogonal data through the simultaneous measurement of redox active and/or ionizable metabolites.

This pilot study showed that this approach has great potential for investigating the effects of disease on the human plasma metabolome.

PCA, readily differentiated contrast from ESRD patients, but could not differentiate between ESRD and ESRD-diabetic patients.

Future research will be directed at developing diagnostic profiles of plasma redox active metabolites that identify ESRD patients at high risk of adverse cardiovascular outcomes, and at determining the utility of diagnostic metabolite profiles for assessing the response to therapies designed to alleviate the uremic syndrome (including dialysis therapies, kidney transplantation, and pharmacologic therapies).

References