

Searching biomarkers for the Complex Regional Pain Syndrome (CRPS) by metabolic profiling of urine using CE/MS

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Introduction

Complex Regional Pain Syndrome (CRPS) is a chronic pain condition characterized by a variety of autonomic, sensory, motor and trophic changes. In most cases CRPS develops after an injury or surgery. However, the pathogenesis of this syndrome remains unclear. As a consequence, diagnosis of CRPS is difficult mainly due to the lack of laboratory tests. The development of reliable diagnostic tests requires clear understanding of biochemical mechanisms of the pathology. Metabolic profiling can provide insight into mechanisms of disease and novel prognostic or diagnostic markers. One of the important clinical manifestations of CRPS is aberrant inflammation and urine could be an appropriate body fluid to access the inflammatory component of disorder.

The described work is part of the TREND (Trauma Related Neuronal Dysfunction) project, for more information please refer to www.trendconsortium.nl

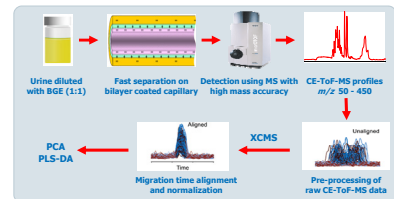


Fig. 1 Schematic of Analytical Workflow

Methods

- CE: Beckman Coulter PA 800 (Fullerton, CA).
- Noncovalently coated fused silica capillary using polybrene and poly(vinylsulfonate): 130 cm x 50 µm ID.
- Background electrolyte (BGE): 1M formic acid pH 1.8.
- Sample injection: hydrodynamic injection with pH-mediated stacking using 12.5% NH₄OH.
- ESI-TOF-MS: MicrOTOF (Bruker Daltonik, Bremen, Germany).
- CE-MS coupling: sheath-flow interface and grounded ESI sprayer.
- Sheath liquid: 50% methanol and 0.1% formic acid.
- Electrospray: positive ionization mode, -4.5 kV ionization voltage.
- Data evaluation: DataAnalysis software (Bruker Daltonik GmbH, Germany) for molecular feature extraction, XCMS (Scripps Center for Mass Spectrometry, La Jolla, CA) and SIMCA-P+ software (Umetrics, Umea, Sweden).
- Real samples: 29 urine samples from patients with chronic CRPS and 18 urine samples from healthy controls.
- Sample preparation: urine 1x diluted with BGE and centrifugated for 5 min at 13200 g.

Results – Analytical Method

Based on previous work on amino acid (AA) profiling using CE-TOF-MS [Mayboroda et. al. J Chrom A 1159 (2007) 149-53], the stability of migration times has been improved by using capillaries dynamically coated with a bilayer of polybrene (PB) and poly(vinyl)sulfonate (PVS). In-capillary pre-concentration using pH-mediated stacking allowed for limits of detection in the nanomolar concentration range. The effect of the NH₄OH concentration in the pre-injection plug on the separation efficiency of valine and phenylalanine is given in Fig 2.A. The separation of an AA standard mixture is presented in figure 2.B using an optimized injection volume of ca. 100 nL (with preinjection of 12.5% NH₄OH solution).

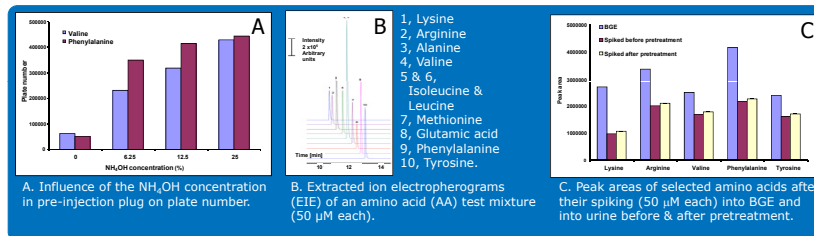


Fig. 2 Optimization of analytical method in terms of separation efficiency and sample preparation.

In order to obtain good focusing, the urine sample has to be acidified prior to injection which is achieved by dilution with BGE (1:1; v/v). The effect of the urine matrix on the signal intensity of selected AAs were investigated by spiking experiments. Figure 2.C depicts the peak areas of selected AAs in BGE and spiked into urine prior and after pretreatment (1x dilution in BGE + centrifugation). A significant decrease of signal intensity is observed for AAs in the urine samples due to ionization suppression. The magnitude of this effect was consistent from one urine sample to another.

Results – Statistical Evaluation

The optimized CE-TOF-MS method was then applied to a set of clinical urine samples of 29 CRPS patients and 18 controls. All data files were recalibrated and exported as netCDF files for further processing. Migration time alignment was performed using XCMS software. The resulting file (TSV data) was exported to SIMCA-P+ 11.2 for further analysis. Initial analysis was performed using principle component analysis (PCA) yielding a considerable grouping first PCs, but with very limited predictive ability (data not shown).

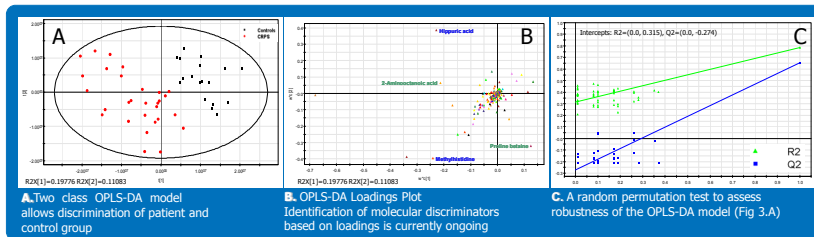


Fig. 3 Statistical Analysis using OPLS-DA.

● ASMS 2008, Poster MPL 298

Partial least square discriminant analysis with orthogonal signal correction (OPLS-DA) applying the known class information was subsequently applied to the data. The OPLS-DA scores plot given in Fig. 3A shows the expected grouping with an initial identification of corresponding loadings in Fig. 3B. R2X(1) and R2X(2) are the Sum of Squares of all the X's explained by the extracted components. The identification of influential loadings is still ongoing and will form the basis for the biological interpretation. In order to judge on the predictive ability of the model, validation by a random permutation test has been performed (Fig. 3C).

Summary

We demonstrated that CE-ESI-TOF-MS is an analytical platform fully amenable for clinical metabolomics studies. The biological and clinical interpretation of this data set is the primary goal of further evaluation.

Conclusions

- CE-TOF-MS using non-covalently coated capillaries can be used for fast and reproducible metabolic profiling of urine with minimal sample pretreatment.
- Metabolic profiling of urine from patients with CRPS by PB-PVS CE-TOF-MS offers potential to advance the understanding of the biochemical basis of CRPS.
- Further studies are required to validate CRPS specificity of compounds responsible for classification.