

CHARACTERIZATION OF DIALYZED PROTEINS IN A MARS SYSTEM

**Marina Gorga^{1,2}, Marina Gay¹, Montserrat Carrascal¹,
Albert Parés² & Joaquin Abian¹**

¹CSIC/UAB Proteomics Laboratory, IIBB-CSIC, Edifici M,
Autonomous University of Barcelona, 08193 Bellaterra;

²Liver Unit, Ciberehd, Hospital Clínic, IDIBAPS, C/ Villarroel 170, 08036 Barcelona.

Molecular Adsorbent Recirculating System (MARS) is a extracorporeal liver support which uses albumin to remove low molecular weight toxins in patients with liver diseases⁽¹⁾. Patient blood is dialyzed across an albumin-impregnated membrane against 20% albumin. This albumin solution recirculates in a closed-circuit that includes Charcoal (AC) and strong anion exchange (SAX) resin columns for albumin regeneration.

In this work we analyzed peptides and proteins absorbed into the SAX-MARS resin column after the treatment in patients with resistant pruritus of chronic cholestasis. As commercial albumin used for dialysis already contains other residual proteins, a control sample was prepared recirculating 20% commercial albumin through a MARS SAX resin column for six hours. Proteins bound to the SAX cartridge were extracted using a stepwise gradient of salt and acetonitrile. Protein extracts were digested by trypsin and GluC. Digested peptides were analyzed by multidimensional liquid chromatography coupled to tandem mass spectrometry (MDLC/MS/MS) using a linear LTQ ion trap equipped with a microESI ion source. MS/MS fragmentation spectra were searched using SEQUEST search engine against the Human UniProt database.

With this method a total of 146 different proteins were identified to be absorbed by the SAX resin column. Forty three proteins were observed only after patient treatments while the rest were already present in the MARS-albumin. Presence of some of these tentative patient-derived proteins was confirmed by Liquid Chromatography and Multiple Reaction Monitoring focusing on the precursor and product ions corresponding to target peptides⁽²⁾. By using this methodology, five proteins have been confirmed to be dialyzed from patients (Neutrophil Defensin 1, Secreted Ly-6/uPAR-related protein 1 (SLUR1), Serum Amyloid A, Fibrinogen Alpha Chain and Pancreatic prohormone precursor). Among these, the presence of Neutrophil Defensin 1 and SLUR1 has also been validated by western blotting.

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