Biocompatibility assessment of haemodialysis membrane materials by proteomic investigations

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OBJECTIVES

The exposure of blood to an artificial surface such as the haemodialysis membrane results in the nearly instantaneous deposition of a layer of plasma proteins. The composition of the protein layer profoundly influences all subsequent events, and to a large extent determines the biocompatibility of the biomaterial [1-7]. In the present study, we examine the protein adsorption capacity and coagulation profiles of the polysulfone-based helixone material in comparison to cellulose triacetate. Shotgun proteomics data-independent analysis was applied to eluates obtained with each membrane after a dialysis session, in order to assess the function of desorbed proteins.

METHODS

We examined and compared the blood protein adsorption patterns and coagulation profiles of two different membrane materials used for clinical haemodialysis in ESRD patients: CTA, a chemically engineered modified cellulosic membrane, and the fully synthetic polymer polysulfone-based HE. Proteomics analysis was carried out on eluates from 3 male non-diabetic haemodialysis patients; all patients had been on regular dialysis treatment and were on a stable intradialytic anticoagulation regimen (unfractionated heparin). Coagulation parameters (prothrombin time, partial thromboplastin time, fibrinogen) were measured before and after the haemodialysis session; Platelet aggregation testing was performed by a multiplate reader (Roche) using the TRAPtest, platelet stimulation via the thrombin receptor (TRAP-6) cascade, and the COLtest, collagen-induced aggregation. GpIIb/IIIa antagonist reagent was applied as a negative quality control.

Graphs and tables

A

ID	Molecules in Network	Score	Top Diseases and Functions
1	AHSG,ALB,AMBP,APOA1,APOA2,APOA4,APOC2,APOC3,APOF,APOH,CLU,**ERK1/2**,GC,**Growthhormone,HDL,HDL-cholesterol,hemoglobin**,HPX,**IGC**,**Ldh (complex),Nr1h**,ORM1,ORM2,**Pro-inflammatory Cytokine,Rbp,**RBP4,**SAA**,SAA1,SAA2,SERPINA1,**Sod**,TF,TTR,**VLDL-cholesterol**,VTN	56	Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry
2	**Akt,Alp,ALT,**APOE,CD14,CFH,CFHR1,CFHR2,**Collagen type\I,Collagen(s),alastase,Fibrinogen,GOT,**HRG,**IgG-IgG 1,Igg3,**IGHG1,IGHG2,IGHG3,IGHG4,IGKC,IGLC1,IGLL1/IGL5,IL1,IL12(complex),Immunoglobulin,Laminin,LDL,LDL-cholesterol,**LRG1,**Mek,P38 MAPK,Tgf beta,**WISP2	32	Cellular Assembly and Organization, Cell Death and Survival, Cardiovascular Disease

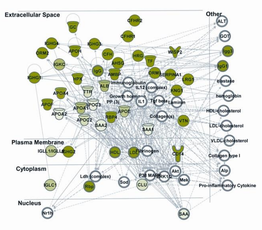

Fig. 1 Ingenuity Pathway Analysis (IPA) of CTA retained proteins pool of dataset. (A) Network table of the two top ranking network and (B) merge of the first two top ranking network generated. Network Focus Molecules are mostly extracellular (secreted) proteins involved in: Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry, Cellular Assembly and Organization, Cell Death and Survival, and Cardiovascular Disease.

A

ID	Molecules in Network	Score	Top Diseases and Functions
1	**ACTA1,ACTA2,ACTC1,ACTG1,ACTG2,Alphaactin,C3,C9,Collagen(s),DPT,ERK1/2,FCN2,FGA,Fibrin,GActin,HBB,HBD,HBE1,HBG1,HBG2,HDL,hemoglobin,Immunoglobulin,Integrin,Mac,MASP1,MASP2,MGP,PRAP1,PRDX2,Pro-inflammatory Cytokine,**SAA1,SAA2,**SERPINC1,**VTN	62	Hematological Disease, Hereditary Disorder, Developmental Disorder
2	**ACTBL2,ALDH1A2,ARIDSB,ASF1B,BARX2,C2orf40,CA1,CCND1,CLCN5,DYNLL1,EPCAM,FHOD1,FUS,GLI52,GUCA2B,HIP1R,HMMR,HSD17B7,LAMA5,LIN28B,LIN7C,MAST1,MEP1A,NRF1,POLR1B,POTEE/POTEF,POTEI,POTEJ,PTH,PXK,S100P,SLC9A3,TAP2,TRAPPC2,UBC**	15	Organismal Injury and Abnormalities, Renal and Urological Disease, Molecular Transport

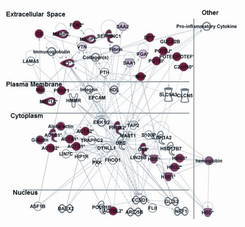

Fig. 2 Ingenuity Pathway Analysis (IPA) of HE retained proteins pool of dataset. (A) Network table of the two top ranking network and (B) merge of the first two top ranking network generated. Network Focus Molecules are both extracellular (secreted) and cytoplasmic proteins involved in: Hematological Disease, Hereditary Disorder, Developmental Disorder, Organism Injury and Abnormalities, Renal and Urological Disease, and Molecular Transport.

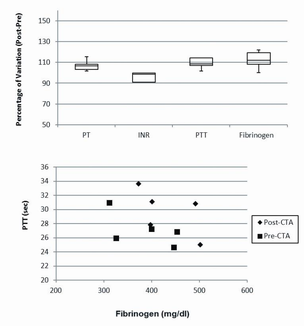

Fig. 3 Percentage variation after-before dialysis session of coagulation parameters (upper panel) and correlations between fibrinogen concentration and partial thromboplastin time (lower panel) in patients undergoing haemodialysis with CTA membrane.

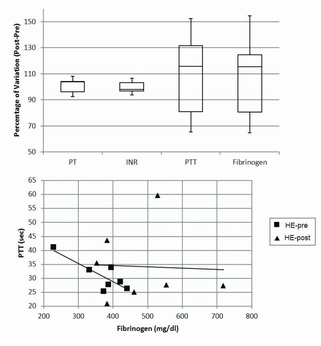

Fig. 4 Percentage variation after-before dialysis session of coagulation parameters (upper panel) and correlations between fibrinogen concentration and partial thromboplastin time (lower panel) in patients undergoing haemodialysis with HE membrane.

RESULTS

In this work, we analysed the biocompatibility of cellulose triacetate (CTA) and polysulfone-based helixone (HE) materials by means of a differential proteomics profiling experiment by data independent analysis based on a shotgun discovery proteomics method. We found an average of 65 proteins differentially adsorbed on the two membranes: most of the proteins retained by CTA belong to the extracellular matrix and extracellular region component and are involved in networks related to lipid metabolism, molecular transport, small molecule biochemistry, cellular assembly and organization, cell death and survival, and cardiovascular disease (Fig. 1 A and B) while HE membrane mostly retains a low abundance of plasma proteins belonging to macromolecular complexes, organelles, cell parts and a low level of proteins belonging to the extracellular matrix or extracellular regions howing their involvement in hematological disease, hereditary disorder, developmental disorder, organism injury and abnormalities, renal and urological disease, and molecular transport (Fig. 2 A and B). Concerning coagulation cascade, fibrinogen chains, serum amyloid proteins, insulin-like growth factor, and inhibin chains are more retained by the HE membrane, as well as for antithrombin and integrin (Fig 3 and 4). These data confirmed the retention on polysulfone-based HE membranes of proteins associated with the coagulation cascade, possibly suggesting an active role by this material in modulating this molecular mechanism.

CONCLUSIONS

Interfacial phenomena taking place on or in the material (adsorption and alteration of proteins) are crucial for biocompatibility and function since they can initiate complex cascade processes in the biological system. Identification of proteins surface-adsorbed onto the dialysis membrane material can thus provide important insights into reactions occurring during the haemodialysis procedure. We show here that the use of proteomic techniques may give valuable molecular and submolecular information on interfacial structures. Improved knowledge about the impact of biomaterial surface parameters upon interaction with plasma patients may hopefully lead to the development of more biocompatible polymers for the potential benefit of the uraemic patient.

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