http://www.kidney-international.org

Specific adsorption of some complement activation proteins to polysulfone dialysis membranes during hemodialysis

Jan Mares[1], Visith Thongboonkerd[2], Zdenek Tuma[3], Jiri Moravec[3] and Martin Matejovic[1]

[1]*Department of Internal Medicine I, Charles University Medical School and Teaching Hospital, Plzen, Czech Republic;* [2]*Medical Proteomics Unit, Office for Research, and Development Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand and* [3]*Proteomic Laboratory, Charles University Medical School, Plzen, Czech Republic*

Dialyser bioincompatibility is an important factor contributing to complications of hemodialysis with well known systemic consequences. Here we studied the local processes that occur on dialysis membranes by eluting proteins adsorbed to the polysulfone dialyser membranes of 5 patients after 3 consecutive routine maintenance hemodialysis sessions. At the end of each procedure, a plasma sample was also collected. These eluates and their accompanying plasma samples were separated by 2-dimensional gel electrophoresis; all proteins that were present in all patients were analyzed by tandem mass spectrometry; and a ratio of the relative spot intensity of the eluate to plasma was calculated. Of 153 proteins detected, 84 were found in all patients, 57 of which were successfully identified by mass spectrometry as 38 components of 23 unique proteins. In 10 spots the relative eluate intensity differed significantly from that in the plasma, implying preferential adsorption. These proteins included ficolin-2, clusterin, complement C3c fragment, and apolipoprotein A1. Our finding of a selective binding of ficolin-2 to polysulfone membranes suggests a possible role of the lectin complement pathway in blood-dialyser interactions.

Kidney International (2009) **76,** 404–413; doi:10.1038/ki.2009.138; published online 6 May 2009

KEYWORDS: biocompatibility; complement; dialyser elution; ficolin; lectin pathway; proteome

Correspondence: *Jan Mares, Department of Internal Medicine I, Charles University Teaching Hospital, Alej Svobody 80, 30460 Plzen, Czech Republic. E-mail: mares@fnplzen.cz*

Received 16 December 2008; revised 11 February 2009; accepted 18 March 2009; published online 6 May 2009

Hemodialyser bioincompatibility has long been established as an important factor in various complications accompanying both chronic and acute hemodialysis (HD). Several aspects of biological response triggered by blood exposure to a dialyser, namely coagulation, complement level, and leukocyte activation, were recognized shortly after the introduction of the artificial dialysis membrane into clinical practice.[1,2] In the past decades, we gathered comprehensive information covering metabolic consequences of these processes; that is, chronic micro-inflammation, enhanced oxidative stress, and pro-coagulant condition.[3–6] Although difficult to link directly with increased mortality, bioincompatibility is held (at least partially) responsible for accelerated atherosclerosis, malnutrition, and thrombotic diathesis found in HD patients.[7–9]

Although we have ample data regarding systemic effects of the interaction between artificial material surface and blood, its molecular substrates remain obscure. Yet, understanding the mechanisms responsible for foreign pattern recognition and launching a subsequent reaction cascade could help us to develop better-tolerated materials, and to prevent the adverse sequelae. Most studies addressing this issue were accomplished under simplified laboratory conditions (by means of phantoms and pre-fractionated plasma),[10–14] or targeted onto a predefined set of molecules.[15–22] However, to investigate the subject in its full complexity and in an unbiased manner, the elution of dialysers used in regular HD and in the proteomic approach is crucial.

Therefore, the aim of our study was to verify the elution algorithm eligible for proteomic analysis, to highlight the eluate proteome, and to qualify the potential molecules involved in the blood–dialyser interaction. To the best of our knowledge, this is the first trial studying with proteomic method the full spectrum of proteins adsorbed by a hemodialyser in a clinical setting.

RESULTS

The protein concentrations of both eluate (acetic acid) (449 (98.9, 2487.3) mg/l) and washout (ethylenediaminetetraacetic acid in phosphate buffered saline, PBS/EDTA) (260.3 (156, 779.4) mg/l) were significantly higher ($P<0.001$) than

in the final 10-ml flush (Plasmalyte) (24.4 (19.2, 27.6) mg/l). The difference in protein concentrations between eluate and washout was not statistically significant ($P = 0.17$). Although the variation in protein content was relatively low in the flush (coefficient of variation, CV = 22%), it was considerably higher in the washout (CV = 152%) and in the eluate (CV = 154%). Nevertheless, protein concentrations were closely correlated in the latter two samples ($r = 0.82$, $P < 0.001$).

In total, 45 gels derived from 15 dialyser eluates and the corresponding plasma samples were analyzed. A representative two-dimensional (2D) gel-scanned image is shown in Figure 1 to demonstrate the typical eluate proteome map. There were 153 spots detected in at least one eluate sample, 84 of them were found in all 5 patients, and 44 were present in all procedures. Protein spots were distributed along the molecular weight (MW) range of 15–175 kDa and pI range of 3.4–8.9. However, 95% of the overall protein spot intensity was localized inside the interval of MW of 18–85 kDa and pI of 3.4–7.7. Besides that, samples of the PBS/EDTA washout from three dialysers were used to prepare another nine gels and compared with corresponding plasma and eluate spot patterns. As all the spots detected in the washout were present in the acetic eluate as well, and the quantitative analysis of relative spot intensities did not show any significant differences between the washout and plasma, only acetic eluates were submitted for further analysis.

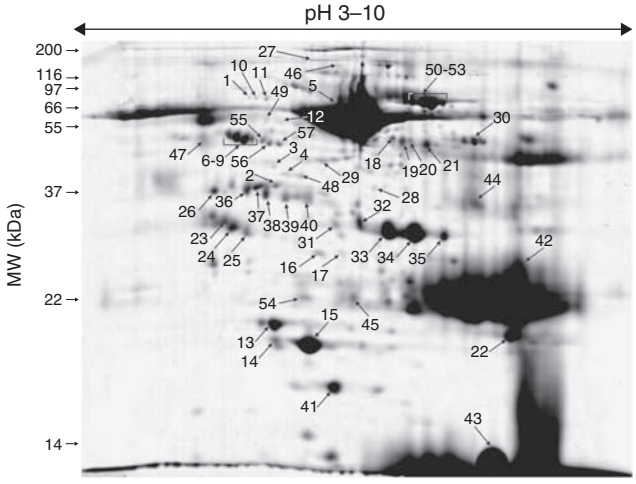

Figure 1 | Representative two-dimensional gel image of dialyser eluate. Spots labeled with numbers were successfully identified by tandem mass spectrometry analyses (see Table 1). MW, molecular weight.

Spot distribution is shown in Figure 2. Spots detected in all patients ($n = 84$) were excised from gels; 57 spots (those labeled with numbers in Figure 1) were successfully identified by mass spectrometry (MS) and tandem mass spectrometry (MS/MS) analyses as 38 components of 23 unique proteins (Table 1). Some of the identified spots contained multiple isoforms of one protein (most likely because of post-translational modifications), for example, ficolin-2 (spot nos. 31–35) or clusterin (spot nos. 23–25). The protein identifications were further verified by the comparison of measured pI and MW (estimated by the coordinates of the spots in gel) with their theoretical values (as predicted by the protein database). Confirmed identifications are marked with an asterisk in Table 1. Discrepant MWs were common particularly in albumin (spot nos. 28, 29, 38–40) and in hemoglobin (spot nos. 30, 42, 44) co-migrated with another protein.

In several protein spots (that is, complement C3, mannose-binding lectin-associated serine proteases (MASP) 1 and 2), the measured MWs were lower than the theoretical ones. This difference could be explained by the enzymatic cleavage of their inactive forms. Figure 3 illustrates peptide fragments and amino acid sequences of these proteins established with MS and MS/MS analyses, and indicates the presence of proteolytic cleavage products rather than full-length or native forms. Moreover, MW and pI measured in 2D gels corresponded better with the theoretical values of their respective fragments: C3 complement (spot no. 26; measured MW: 37 kDa, pI: 4.8) vs C3c α-chain fragment 2 (theoretical MW: 39.5 kDa, pI: 4.8); MASP-1 (spot no. 47; measured MW: 44 kDa, pI: 4.0) vs MASP-1 heavy chain (theoretical MW: 49 kDa, pI: 4.9); and MASP-2 (spot no. 48; measured MW: 39 kDa, pI: 5.5) vs MASP-2A (theoretical MW: 47.7 kDa, pI: 5.4).

The variability of eluate intensities of the corresponding spots among patients (CV inter-individual) ranged from 6 to

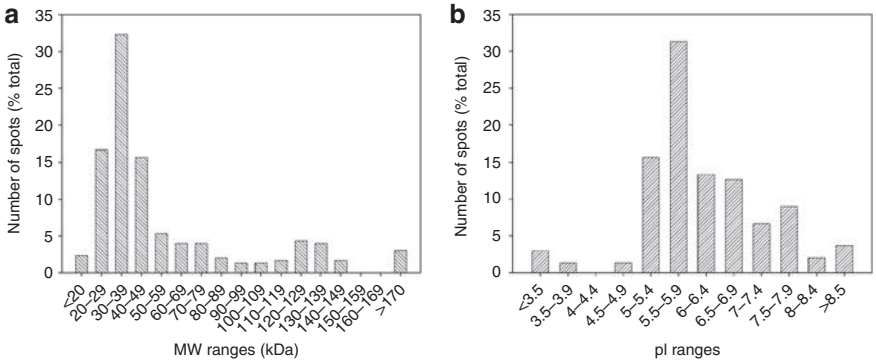

Figure 2 | Basic characteristics of proteins detected in dialyser eluate. (a) Molecular weight (MW) and **(b)** pI distributions of proteins in dialyser eluate. Percentage per category of total spot number are given.