Hemodialysis International 2006; **10**: S2–S4

Original Articles

Removal of uremic plasma factors using different dialysis modalities

Mario BONOMINI,[1] Enrico BORDONI,[2] Francesca CIABATTINI,[2] Tiziana DI MARCO,[3] Mario LIANI,[2] Luigi FANTETTI,[4] Gennaro MACCHIAGODENA,[4] Bernardina MANCINI,[5] Carlo NUSCA,[6] Sergio VECCHIOTTI,[6] Vittorio SIROLLI[1]

[1]University of Chieti-Pescara, [2]Hospital of Fabriano (AN), [3]Hospital of Penne (PE), [4]Hospital of Larino (CB), [5]Hospital of L'Aquila, [6]Hospital of Avezzano (AQ), G. d'Annunzio University, Chieti-Pescara, Italy

Key words: Hemodialysis, online hemodiafiltration, uremic toxins, phosphatidylserine, β_2-microglobulin, erythrocyte

INTRODUCTION

Uremia is a complex clinical syndrome that resembles a systemic "poisoning," caused by retention of a myriad toxic substances because of inadequate kidney function. On the basis of their molecular weight (MW), uremic retained solutes can be schematically classified into 3 main groups: (1) low-MW molecules (< 300 Da), which can be subdivided as water-soluble (nonprotein bound) compounds such as urea and creatinine, and protein-bound solutes, e.g., p-cresol and hippuric acid, (2) middle-MW range compounds (300–12,000 D), like β_2-microglobulin, peptide-linked advanced glycosylation end-products (AGEs), and parathyroid hormone, and (3) high-MW solutes (> 12,000 Da) such as leptin and complement factor D.

The contribution of medium-high-MW substances to uremic pathophysiology has been increasingly recognized, and has promoted interest in using high permeability membranes and convective treatment modalities, as convection plays a major role in the transport mechanism of large solutes.[1] Hemodiafiltration with online (OL) production of substitution fluid (online-high-flux hemodiafiltration (OL-HDF)) combines diffusion and convection features in a single mode of treatment to provide solute removal over a wide range of MWs. It is a safe, well-tolerated technique that seems to offer an optimal form of extracorporeal treatment for the dialysis patient.

The advantage of hemodialysis (HD), viz. high removal of small solutes such as urea and creatinine by diffusive processes, is maintained in HDF if the membrane composition, blood flow, and dialysate flow are constant.[2] The mass removal of phosphate during postdilutional online HDF, however, may be greater than in HD[3] and allow a better control of calcium-phosphorus homeostasis in the medium term.[4]

On the other hand, a considerable difference exists between HD and HDF in the MW spectrum of substances preferentially removed. In contrast with HD, the convective component of OL-HDF allows the removal of medium-MW and large-MW solutes, with improved clearances up to a postdilution substitution volume of 100 mL/min.[5] Several in vivo studies have shown that with OL-HDF removal of β_2-microglobulin improves.[6–8] The elimination of β_2-microglobulin, the yardstick for measuring the ability and efficiency of removing large solutes, has as its main objective the reduction of the predialysis β_2-microglobulin level as this may postpone the onset of dialysis-related amyloidosis. Predialysis β_2-microglobulin levels in OL-HDF proved to be significantly lower than in low-flux HD after 3 months of treatment, and this difference persisted over a 2-year study period.[6]

Compared with high-flux HD, an increased removal capacity was also shown in HDF for osteocalcin (a medium-molecule marker) and myoglobin (a large-molecule

Correspondence to: M. Bonomini, MD, Clinica Nefrologica—Emodialisi, Ospedale Clinicizzato "SS. Annunziata," Via dei Vestini, 66013 Chieti, Italy.
E-mail: m.bonomini@nephro.unich.it

marker),[8] complement fractions such as factor D,[9] and asymmetric dymethyl-arginine.[10] Serum levels of tumor necrosis factor-α, a cytokine linked to the inflammatory response, as evaluated in 24 patients dialyzed with the same membrane (polysulfone), increased during low-flux HD, but decreased during the high-flux HD session and decreased even more during OL-HDF.[11] Moreover, it has recently been observed that postdialysis serum levels of AGEs are significantly lower in patients treated with on-line HDF than in those treated with conventional HD, despite similar predialysis levels.[12] Advanced glycosylation end-products are retained in uremia, up to a 10-fold increased concentration,[13] and are held responsible for tissue damage and functional disturbances. The mean serum AGE level reduction rate in OL-HDF (61.5%) was significantly higher than in conventional HD (20.5%; $p < 0.001$) and high-flux HD (40.4%; $p < 0.05$).[12]

The evidence available suggests that OL-HDF offers the best clearance profile currently available in chronic replacement therapy,[2] including the removal of larger solutes, which is considered an important target for improving dialytic performance. Long-term observations are necessary to evaluate the clinical relevance of such solute removal capabilities with OL-HDF.

Removal by different dialysis modalities of uremic plasma factor(s) causing loss of erythrocyte membrane phospholipid asymmetry

The phospholipids of the human red blood cell (RBC) are distributed asymmetrically in the bilayer of the erythrocyte plasma membrane, with aminophospholipid phosphatidylserine (PS) normally located in the membrane's inner leaflet. Maintenance of this asymmetry is an energy-requiring process of major physiological importance for the cell. Indeed, exposure of PS on the outer leaflet of the erythrocyte membrane may have several pathophysiological implications, including generation of a signal that triggers macrophage recognition, promotion of a hypercoagulable state through the formation of the prothrombinase complex, and an increased erythrocyte propensity for adhesion to the endothelium.[14]

We observed that chronic renal failure is associated with retention of compound(s) which cause increased exposure of PS on the outer surface of the erythrocyte membrane.[15] The phenomenon seems related to the inhibition of PS transport from the outer to the inner leaflet of the erythrocyte membrane, and is strongly affected by RBC exposure to uremic plasma. Normal erythrocytes incubated in plasma from HD patients show an increase in PS-expressing red cells, reaching values comparable with those found in uremic patients, while uremic RBCs re-incubated in normal plasma show a decrease in the percentage of PS positivity.[15] Preliminary in vitro experiments indicate that the putative uremic compound(s) causing RBC PS exposure are heat unstable, highly lipophilic, somehow associated with plasma proteins, and with a MW between 10 and 20 kDa.[15,16] Little, however, is known about whether and to what extent dialysis removes such plasma factor(s).

To address such an issue, we recently conducted a prospective, randomized crossover study to examine the effects of various different dialysis modalities on the removal of the circulating uremic factor(s) causing increased RBC PS externalization.[16] Each patient included in the study was treated with HD and with OL-HDF using high-flux polysulfone or the new polysulfone-based high-flux helixone membranes. Removal was assessed indirectly by measurement of PS-expressing normal RBCs exposed to uremic plasma or ultrafiltrate obtained during the extracorporeal session.

The reduced ability of uremic plasma at the end of dialysis to cause RBC PS exposure and the capacity of ultrafiltrate to greatly increase the percentage of PS-positive normal erythrocytes demonstrate that removal of the uremic plasma factor(s) causing RBC PS exposure occurred under the selected dialytic conditions of the study. The degree of removal, however, varied according to the different experimental conditions. Removal was greater for OL-HDF using the helixone membrane, intermediate with HD using the helixone and with OL-HDF using standard polysulfone, and lower with HD using standard polysulfone membrane.[16] These results confirm the ability of OL-HDF to eliminate high-MW compounds, particularly when using helixone, a membrane designed specifically for the removal of uremic toxins in the range of β_2-microglobulin (11,800 Da) while maintaining a low albumin sieving coefficient.[17]

Increased exposure of PS on the outer surface of RBC membrane may be involved in the pathophysiology of uremia by promoting abnormal erythrocyte interactions. Surface-exposed PS enhances the susceptibility of uremic RBCs to phagocytosis,[18] and may thus represent a possible pathogenic mechanism behind cell removal in a condition associated with shortened erythrocyte survival, as in uremia. In addition, increased PS exposure in uremic erythrocytes may facilitate the activation of the hemostatic system and have a part in inducing a hypercoagulable state in uremia.[19] Finally, surface-exposed PS causes an enhanced adhesion of uremic erythrocytes to the vascular

endothelium, which may inhibit endothelial nitric oxide synthase (eNOS), the enzyme that continuously synthesizes nitric oxide. We observed that PS-mediated increased adherence of uremic RBCs to endothelial cultures may cause a decrease in the levels of eNOS mRNA and protein, and a significant reduction in eNOS activity,[20] which may offer one explanation for the decreased nitric oxide production in uremia. This mechanism might contribute to endothelial dysfunction and may play a role in the increased atherosclerosis and cardiovascular morbidity of dialysis patients.

From these observations it is possible to hypothesize that removal of the putative soluble uremic compound(s) causing abnormal erythrocyte membrane PS externalization may benefit dialysis patients. A multicenter clinical study dealing with the effects of reducing RBC PS exposure is in progress to determine whether this concept holds true.

REFERENCES

1 Locatelli F, Manzoni C, Di Filippo S. The importance of convective transport. *Kidney Int*. 2002; **61**:S115–S120.
2 Spalding E, Farrington K. Haemodiafiltration: Current status. *Nephron Clin Pract*. 2003; **93**:c87–c96.
3 Zehnder C, Gutzwiller JP, Renggli K. Hemodiafiltration—a new treatment option for hyperphosphatemia in hemodialysis patients. *Clin Nephrol*. 1999; **52**:152–159.
4 Minutolo R, Bellizzi V, Cioffi M, et al. Postdialytic rebound of serum phosphorus: Pathogenetic and clinical insights. *J Am Soc Nephrol*. 2002; **13**:1046–1054.
5 Leypoldt JK. Solute fluxes in different treatment modalities. *Nephrol Dial Transplant*. 2000; **15**(Suppl 1):3–9.
6 Wizemann V, Lotz C, Techert F, Uthoff S. On-line hemodiafiltration versus low-flux haemodialysis. A prospective randomized study. *Nephrol Dial Transplant*. 2000; **15**(Suppl 1):43–48.
7 Lornoy W, Because Y, Billiouw JM, Sierens L, van Malderen P. Remarkable removal of beta-2-microglobulin by on-line hemodiafiltartion. *Am J Nephrol*. 1998; **18**:105–108.
8 Maduell F, Navarro V, Cruz C, et al. Osteocalcin and myoglobin removal in on-line hemodiafiltration versus low- and high-flux hemodialysis. *Am J Kidney Dis*. 2002; **40**:582–589.
9 Ward RA, Schmidt B, Hullin J, Hillebrand GF, Samtleben W. A comparison of on-line hemodiafiltration and high-flux hemodialysis: A prospective clinical study. *J Am Soc Nephrol*. 2000; **11**:2344–2350.
10 Schroder M, Riedel E, Beck W. Increased reduction of dimethilarginines and lowered interdialytic blood pressure by the use of biocompatible membranes. *Kidney Int*. 2001; **59**(Suppl 78):19–24.
11 Gil C, Lucas C, Possante C, et al. On-line haemodiafiltration decreases serum TNFα levels in haemodialysis patients. *Nephrol Dial Transplant*. 2003; **18**:447–448.
12 Lin CL, Huang CC, Yu CC, Yang HY, Chuang FR, Yang CW. Reduction of advanced glycation end product levels by on-line hemodiafiltration in long-term hemodialysis patients. *Am J Kidney Dis*. 2003; **42**:524–531.
13 Odetti P, Cosso L, Pronzato MA, Dapino D, Gurreri G. Plasma advanced glycosylation end-products in maintenance haemodialysis patients. *Nephrol Dial Transplant*. 1995; **10**:2110–2113.
14 Zwaal RFA, Schroit AJ. Pathophysiologic implications of membrane phospholipid asymmetry in blood cells. *Blood*. 1997; **89**:1121–1132.
15 Bonomini M, Sirolli V, Settefrati N, Dottori S, Di Liberato L, Arduini A. Increased erythrocyte phosphatidylserine exposure in chronic renal failure. *J Am Soc Nephrol*. 1999; **10**:1982–1990.
16 Bonomini M, Ballone E, Di Stante S, et al. Removal of uraemic plasma factor(s) using different dialysis modalities reduces phosphatidylserine exposure in red blood cells. *Nephrol Dial Transplant*. 2004; **19**:68–74.
17 Ronco C, Bowry S. Nanoscale modulation of the pore dimensions, size distribution and structure of a new polysulfone-based high-flux dialysis membrane. *Int J Artif Organs*. 2001; **24**:726–735.
18 Bonomini M, Sirolli V, Reale M, Arduini A. Involvement of phosphatidylserine exposure in the recognition and phagocytosis of uremic erythrocytes. *Am J Kidney Dis*. 2001; **37**:807–814.
19 Bonomini M, Sirolli V, Merciaro G, et al. Red blood cells may contribute to hypercoagulability in uraemia via enhanced surface exposure of phosphatidylserine. *Nephrol Dial Transplant*. 2005; **20**:361–366.
20 Bonomini M, Pandolfi A, Di Pietro N, et al. Adherence of uremic erythrocytes to vascular endothelium decreases endotehlial nitric oxide synthase expression. *Kidney Int*. 2005; **67**:1899–1906.