JOURNAL OF PROTEOMICS 74 (2011) 2441–2452

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Oxidative stress-associated shape transformation and membrane proteome remodeling in erythrocytes of end stage renal disease patients on hemodialysis

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ARTICLE INFO

Available online 15 April 2011

Keywords:
End stage renal disease
Hemodialysis
Red blood cell
Membrane proteome
ROS production
Oxidative stress

ABSTRACT

This study was designed to evaluate the oxidative stress status of erythrocytes and its association with cellular ultrastructure and membrane proteome modifications in patients with end stage renal disease (ESRD) on hemodialysis (HD). For that purpose, we studied red blood cells' (RBCs) modifications in twelve non-diabetic ESRD patients that were responsive in erythropoietin therapy. Intracellular ROS levels were measured by fluorometry, RBCs ultra-structure was examined by electron microscopy, while the membrane proteome by electrophoresis and immunoblotting. Compared to the healthy subjects, the uremic RBCs exhibited significantly increased ROS accumulation. Dialysis partially ameliorated the basal ROS levels but triggered cellular sensitivity to exogenous oxidative stimuli. Common membrane modifications involved loss, aggregation, fragmentation and carbonylation of critical components as well as over-expression of stress markers. HD significantly contributed to membrane proteome remodeling, especially for aquaporin-1, peroxiredoxin-2 and ubiquitinated proteins. The intracellular redox status and the closely associated membrane modifications seemed to be related to membrane instability, loss of surface area through vesiculation, echinocytosis and stomatocytosis. Our data evinced a network of interactions among the uremic toxins, the RBCs membrane composition and the cellular shape modifications in ESRD, which is developed around a core of oxidative provocations and cellular responses.

1. Introduction

Anemia is a common complication in end-stage renal disease (ESRD) patients on hemodialysis (HD), often leading to higher morbidity and mortality. Inadequate production of erythropoietin, impaired response of erythroid stem cells to erythropoietin, chronic hemolysis and blood loss are leading factors of anemia in ESRD. Furthermore, the RBCs of hemodialyzed

Abbreviations: ESRD, end stage renal disease; Hb, hemoglobin; HD, hemodialysis; MW, molecular weight; PCI, proteome carbonylation index; Prx-2, peroxiredoxin-2; RBCs, red blood cells; SEM, scanning electron microscopy; TEM, transmission electron microscopy; t-BHP, tert-butyl hydroperoxide.

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doi:10.1016/j.jprot.2011.04.009

patients are mechanically stressed by the flow through the dialyzer and the peristaltic pumps as well as metabolically stressed by the accumulation of uremic toxins and the loss of glucose. Due to their reduced resistance to mechanical, metabolic and osmotic stress RBCs lifespan may be shortened [1]. Defected deformability of RBCs that are negatively affected by the HD sessions has also been reported in ESRD patients, compromising both, their rheology in the microvasculature and their survival [2]. Modifications in RBCs membrane protein composition and function may account for the diminished deformability of the ESRD RBCs. Indeed, there is strong evidence for both, damages in several proteins and also for inadequate protein repair in uremic RBCs [3]. Reactive oxygen species (ROS) released by phagocytes and platelets during the contact of blood with the dialysis membranes consist another stressful factor for RBCs in ESRD patients on HD. Disintegration of damaged RBCs and release of hemoglobin (Hb) may further aggravate the pro-oxidant status in the plasma. Moreover, depletion of antioxidants, accumulation of uremic toxins, advanced age, chronic inflammatory state, deficiency of vitamins C, E and selenium and finally, factors connected with HD trigger further accumulation of pro-oxidant compounds in the blood of chronic renal failure patients. Consequently, blood oxidative stress has been established as an intrinsic component of the uremic state [4]. Although many studies have focused on serum markers of oxidation [4,5], the oxidative defects of RBCs membrane represent a primary cause of chronic hemolysis in ESRD patients receiving HD therapy [6]. Indeed, increase in membrane fluidity and cellular osmotic fragility, disturbance of cytoskeleton protein–protein interactions [7] and increased susceptibility to complement deposition [8] have been documented in the context of oxidative stress-associated changes in ESRD erythrocytes.

The aim of the present study is the evaluation of the endogenous ROS accumulation in RBCs and its probable connection with RBCs structural and protein modifications in non-diabetic ESRD patients on HD. Our current data indicate a strong association of corpuscular oxidative and carbonyl stress with several cellular distortions in ESRD, ranging from RBCs shape modifications to membrane proteome defects and stress responses.

2. Materials and methods

2.1. Material supplies

Monoclonal antibodies against Band 3 (B9277) and actin (A5316), polyclonal antibodies against spectrin (S1515) and human IgGs (A8792) and HRP-conjugated antibodies to goat IgGs (A-5420), as well as the Protease Inhibitor Cocktail, t-butyl hydroperoxide (t-BHP) and common chemicals and buffers were all obtained from Sigma-Aldrich (Munich, Germany). Polyclonal antibodies against Hb (GR800GAP) and peroxiredoxin-2 (Prx-2, SP5464) were obtained from Europa Bioproducts (Cambridge, UK) and from Acris GmbH (Hiddenhausen, Germany), respectively. Primary antibodies against CD47 (sc-25773), HSP70 (sc-1060), Fas (Apo-1, sc-715), calpain-1 (μ-calpain, sc-7531) and Band 3 (sc-20657) were from Santa Cruz Biotechnology (Santa Cruz, CA). mAbs to ubiquitinated proteins (#3936) and aquaporin 1 (MCA2099) were obtained from Cell Signaling Technology (Beverly, MA) and AbD Serotec, respectively. mAbs against synexin (annexin VII, 610669) and flotillin-2 (610384) were obtained from BD Transduction Laboratories (San Diego, CA). 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate, acetyl ester (CM-H_2DCFDA) was from Invitrogen, Molecular Probes (C-6827). HRP-conjugated antibodies to rabbit IgGs (NA 934) and ECL Western blot detection kit were from GE Healthcare Amersham (Little Chalfont, Buckinghamshire, UK). HRP-conjugated antibodies to mouse IgGs (P0161) were from DakoCytomation (Glostrup, Denmark). The Oxyblot® detection kit (S7150) was obtained from Millipore, Chemicon (Temecula, CA). Bradford protein assay was obtained from Bio-Rad (Hercules, CA). Gel Analyzer v.1.0 image-processing system and software was obtained from Biosure (Athens, Greece). mAb against stomatin and antiserums against proteins 4.1R and pallidin (band 4.2) were kindly provided by Prof. R. Prohaska (Department of Medical Biochemistry, Medical University of Vienna, Austria) and Prof. J. Delaunay (Service d' Hématologie, Hôpital de Bicetre, Le Kremlin-Bicetre, France) respectively.

2.2. Subjects

We evaluated twelve ESRD patients (Table 1) on standard HD therapy (thrice weekly) and erythropoietin treatment to reach

Table 1 – Demographic characteristics, hematological and serum biochemical data for healthy subjects and ESRD patients.

	Controls (n=12)	ESRD patients (n=12)
Age (years)	45.0±11.5	65.8±13.9[a]
Gender (M/F)	7/5	8/4
Time on HD (months)	–	50±11
WBCs ($\times10^9$/L)	5.78±1.34	6.30±2.16
RBCs ($\times10^{12}$/L)	5.09±0.44	4.00±0.67[a]
Hb (g/dL)	15.81±0.23	11.79±0.72[a]
HCT (%)	46.90±1.26	35.32±1.35[a]
MCV (fL)	92.14±2.13	94.59±7.96
MCV (fL) post-HD	–	**94.89±7.97**
MCH (pg)	31.06±1.11	32.12±1.09
MCHC (g/dL)	33.71±2.15	30.46±4.25
RDW-CV (%)	13.41±0.39	16.32±1.48[a]
RDW-CV (%) post-HD	–	**16.48±1.45**
PLTs ($\times10^3$/μL)	258.22±23.45	236.17±48.22
Glucose (mgr/dL)	85.21±5.78	100.87±13.45
Urea (mg/dL)	29.11±3.45	162.83±34.44[a]
Urea (mg/dL) post-HD	–	**54.99±16.09**[a]
Creatinine (mg/dL)	0.74±0.21	9.56±2.31[a]
Creatinine (mg/dL) post-HD	–	**6.08±2.31**[a]
Cholesterol (mg/dL)	138.34±19.56	153.63±34.31
Uric acid (mg/dL)	4.64±1.26	6.55±2.98[a]
Triglycerides (mg/dL)	150.31±34.96	178.91±65.45
Potassium (mEq/L)	4.32±0.36	5.56±1.72[a]
Potassium (mEq/L) post-HD	–	**4.02±0.76**[b]
Iron (μg/dL)	103.34±12.31	74.11±20.57
Calcium (mg/dL)	9.46±0.21	9.38±0.24
Phosphorus (mg/dL)	3.54±1.11	5.55±1.65[a]

Post-HD measurements are presented in bold. Results are presented as mean±SD.
[a] $p<0.05$ vs. controls.
[b] $p<0.05$ vs. pre-HD.