Activation of granulocytes during hemodialysis: Effects of different dialyzer membranes

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ACTIVACION DE LOS GRANULOCITOS DURANTE LA HEMODIALISIS: EFECTOS DE DIFERENTES MEMBRANAS DE DIALISIS

RESUMEN

Se estudiaron las variaciones plasmáticas de los principales componentes granulocitarios: elastasa, mieloperoxidasa y lactoferrina en más de 200 pacientes con insuficiencia renal crónica en programa de hemodiálisis periódicas. Estos pacientes estaban siendo tratados con diferentes tipos de membranas: cuprofán, policarbonato, celulosa modificada, etilen-vinil-alcohol, polimetilmetacrilato, poliacrilonitrilo y polisulfona. La elevación de los niveles plasmáticos de elastasa y lactoferrina fueron independientes del grado de neutropenia y de la formación de anafilotoxinas. No se encontraron diferencias significativas en las cifras plasmáticas de mieloperoxidasa con las diferentes membranas estudiadas, indicando que ningún dializador es realmente «biocompatible». Los niveles plasmáticos de los constituyentes granulocitarios dependen de la superficie y la composición geométrica del dializador. Nuestros datos indican que durante la diálisis existe una degranulación de los gránulos específicos y/o azurófilos de los polimorfonucleares, incluso en ausencia de activación del complemento.

Palabras clave: ***Biocompatibilidad. Elastasa. Hemodiálisis. Lactoferrina. Mieloperoxidasa.***

SUMMARY

Plasma levels of the main granulocyte components elastase, myeloperoxidase and lactoferrin were investigated in more than 200 patients undergoing regular hemodialysis with dialyzers made from cuprophan, polycarbonate, cellulose hidrate, modified cellulose, ethylene-vinyl alcohol copolymer, polymethylmethacrylate, polyacrylonitrile and polysulfone. Plasma levels of elastase and lactoferrin increased independently of the initial granulocytopenia and anaphylatoxin formation. There were no significant differences between plasma myeloperoxidase levels during dialysis with different membranes indicating that no dializer is truly «biocompatible». Plasma levels of granulocyte constituents depend on the geometry and the surface area of the dialyzer used. Our data indicate degranulation of azurophilic and/or specific granules even in the absence of complement activation.

Key words: ***Polymorphonuclear leukocytes. Elastase. Lactoferrin. Myeloperoxidase. Alpha$_1$-proteinase inhibitor. Hemodialysis. Biocompatibility.***

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Introduction

Three types of neutral proteinases are known to exist in azurophil granules of human polymorphonuclear leukocytes: elastase, which was first described by Janoff and Scherer [1], collagenase, which was found by Lazarus et al [2], and a chymotrypsin-like proteinase (cathepsin G), which was characterized by Rindler et al [3]. Azurophil granules also contain the microbicidal enzymes myeloperoxidase and lysozyme and a variety of digestive enzymes acting both at acid and at neutral pH. Quite in contrast to the azurophiles, the specific granules remain largely uncharacterized. Their constituents are lysozyme, lactoferrin, metalloproteinases acting on collagen, and a vitamin B_{12}-binding protein (for review see Baggiolini et al [4] and Havemann and Gramse [5]).

A profound but transient granulocytopenia has well been documented during the initial phases of hemodialysis with cellulosic membranes [6-12]. Such leukopenia results from pulmonary sequestration of leukocytes provoked by complement-derived fragments [13-16]. Active components of C3 and C5 act as anaphylatoxins and chemotoxins; they also have the property of inducing release of neutrophil granule material independent of phagocytosis [17, 18]. On the other hand, it is evident that neutrophils have constituents that will interact indirectly and directly with the complement system resulting in complement activation and cleavage of complement proteins [19, 20]. This report summarizes data concerning the potential role of different dialyzer membrane materials on the plasma levels of main granulocyte component in patients undergoing regular hemodialysis treatment.

Methods

Patients

More than 200 chronically uremic patients undergoing regular hemodialysis treatment were studied. Hemodialysis was performed three times weekly using different dialyzers made from cuprophan (Gambro Fiber GF-120-H, GF-180-H, Gambro Lundia Plate 1.0 and 1.36, Gambro, Hechingen, FRG; MTS C 1.2, MTS, St. Wendel, FRG; Hemoflow D_2, Fresenius, Oberursel, FRG), polymethylmethacrylate (Filtryzer 1.56, Toray, Tokyo, Japan), ethylene-vinyl alcohol copolymer (KF 101, Salvia, Hamburg, FRG), polyacrylonitrile (Biospal 2400 S, Hospal, Lyon, France), polysulfone (F40, Fresenius, Oberursel, FRG), polycarbonate (FD 100, Gambro, Hechingen, FRG) and modified cellulose (Hemophan, Enka AG, Wuppertal, FRG).

Sampling procedures

Whole blood samples were drawn from the arterial side of the patient's arteriovenous fistula after heparinization prior to and during hemodialysis. All blood samples were anticoagulated with EDTA, centrifuged and the plasma was separated immediately to prevent leakage of leukocyte constituents. The specimens were stored at −30° C and were thawed only once for processing.

Assay procedures

The measurement of plasma levels of the granulocyte elastase in complex with alpha$_1$-proteinase inhibitor (E-alpha$_1$PI) was performed with a highly sensitive enzyme-linked immunoassay as described by Neumann et al [21]. Plasma levels of granulocyte lactoferrin and myeloperoxidase were assayed with highly sensitive enzyme-linked immunoassays as previously described [22].

Statistics

All values are given as mean ± SEM. For multiple comparisons of data obtained at different time points, an analysis of variance (ANOVA) was used. The significances of differences between the study periods were analyzed with Wilcoxon's test for paired samples.

Results

The effect of hemodialysis therapy on plasma E-alpha$_1$PI, myeloperoxidase and lactoferrin is shown in table I. Cellulose hydrate membrane caused a maximum E-alpha$_1$PI concentration of 1,659.0 ± 256.8 ng/ml ($p < 0.001$). The increase of plasma E-alpha$_1$PI value was also significant after 120 min of hemodialysis ($p < 0.01$) in comparison to all other membranes. Cuprophan membrane induced an E-alpha$_1$PI increase of 388.1 ± 51.6 ng/ml, whereas maximal E-alpha$_1$PI values of 643.0 ± 174.7 ng/ml were measured in the presence of the polymethylmethacrylate membrane. Both membranes caused significantly higher E-alpha$_1$PI levels at the end of hemodialysis compared with the polyacrylonitrile or polysulfone membrane. Plasma E-alpha$_1$PI values were 381.9 ± 54.0 ng/ml at the end of dialysis using the ethylene-vinyl alcohol copolymer membrane, and this increase was only statistically different from the values obtained in the presence of cellulose hydrate membrane. Polysulfone membrane caused the lowest lactoferrin levels, whereas plasma myeloperoxidase levels were unchanged comparing the effects of different dialyzer membranes.

Plasma levels of E-alpha$_1$PI and lactoferrin were followed during hemodialysis in patients dialyzed with cuprophan membranes and analyzed with respect to the configuration and membrane surface area of the dialyzer (table I). As can be seen, flat sheet dialyzers caused higher plasma levels of E-alpha$_1$PI than hollow fiber dialyzers. Moreover, a second phenomenon could be observed, namely that dialyzers with smaller

Table I. Effect of different membrane materials on plasma levels of granulocyte elastase in complex with alpha$_1$-proteinase inhibitor (E-alpha$_1$PI), myeloperoxidase and lactoferrin at the start and the end of hemodialysis.

	E-alpha$_1$PI (ng/ml)		Myeloperoxidase (ng/ml)		Lactoferrin (ng/ml)	
	Start	End	Start	End	Start	End
PMMA Filtryzer 1.5 (n = 10)	114.2 ± 18.1	681.8 ± 102.6	53.9 ± 9.7	178.3 ± 27.1	166.6 ± 28.5	712.5 ± 165.9
Polysulfone F40 (n = 8)	80.6 ± 8.4	200.3 ± 33.2	31.6 ± 3.5	97.2 ± 22.0	76.6 ± 13.9	192.0 ± 33.3
PAN Hospal 2400 S (n = 7)	74.9 ± 8.1	267.6 ± 79.6	42.4 ± 15.4	136.9 ± 48.5	122.6 ± 23.5	647.7 ± 203.6
Polycarbonate FD 100 (n = 10)	125.8 ± 14.4	525.3 ± 94.3	31.8 ± 3.8	140.1 ± 33.0	61.1 ± 5.2	327.9 ± 81.1
Cuprophan GF 120 H (n = 10)	107.3 ± 11.5	388.1 ± 51.6	48.8 ± 5.7	161.3 ± 24.8	64.9 ± 8.2	346.6 ± 135.7
Cellulose hydrate Second (n = 10)	207.3 ± 58.4	1,6590 ± 256.8	n.d.	n.d.	n.d.	n.d.
EVAL KF 101 (n = 10)	142.2 ± 23.9	381.9 ± 54.0	n.d.	n.d.	n.d.	n.d.

Mean values ± s.e.m.; n.d. = not done.

surface areas caused lower plasma levels of E-alpha$_1$PI as compared to dialyzers with larger surface areas. This held true for both hollow fiber and flat sheet dialyzers. Similar results were obtained measuring plasma lactoferrin values (table II).

Table II. Effect of different cuprophan dialyzers on plasma levels of granulocyte elastase (E-alpha$_1$PI) and lactoferrin at the end of hemodialysis

	E-alpha$_1$PI (ng/ml)	Lactoferrin (ng/ml)
Hemoflow D$_2$ (n = 10)	682 ± 103	418 ± 07
Gambro Lundia Plate:		
1.0 m^2 (n = 12)	650 ± 75	910 ± 85
1.36 m^2 (n = 6)	1,395 ± 135	1,290 ± 135
GF-120 H (n = 10)	526 ± 48	740 ± 65
Hemophan (n = 10)	426 ± 40	666 ± 51

Mean values ± s.e.m.

Discussion

Leukocyte activation during hemodialysis is associated with the release of lysozyme [23] and lactoferrin [24, 25]. Ivanovich et al [26] reported an increase in lactoferrin levels during hemodialysis with cuprophan and, to a lesser extent, cellulose acetate. Hällgren et al [25] found increased levels in lactoferrin and eosinophilic cationic protein during dialysis with cuprophan membranes. On the other hand, appreciable membrane binding of lactoferrin and lysozyme has been reported [27]. Predialysis lysozyme levels were 3 to 4 times normal in both hemodialysis and CAPD patients [25, 28]. Patients dialyzed with cellulose hydrate, cuprophan, or ethylenevinyl alcohol copolymer dialyzers failed to exhibit significant changes of plasma lysozyme values. In contrast, there was a significant decrease of plasma lysozyme concentration during and immediately after hemodialysis in the presence of polyacrylonitrile and polymethylmethacrylate membranes [28]. Two other studies also found that lysozyme levels in the afferent blood did not rise [14, 25].

We observed a progressive elevation of plasma E-alpha$_1$PI during hemodialysis therapy using dialyzers made from cuprophan [29]. Plasma E-alpha$_1$PI levels were higher in diabetics compared with nondiabetic dialysis patients [30]. When we studied the effect of different dialyzer membranes on the release of granulocyte elastase, marked differences were observed [12, 28]. Similar results were obtained by Knudsen and coworkers [31]. The modified cellulosic membrane (Hemophan ®) caused lower release of granulocyte elastase and less leukopenia than the regenerated cellulose (Cuprophan ®) [32]. If we compare the results with different membrane materials there is no relationship between initial leukopenia and increased E-alpha$_1$PI levels [12, 28]. Bonal et al [33] also found no correlation between increased elastase levels and decrease of total leukocyte count.

92 % of human granulocyte elastase is bound by alpha$_1$-antitrypsin (alpha$_1$-proteinase inhibitor) and 8 % by alpha$_2$-macroglobulin [34]. The chymotrypsin-like proteinase (cathepsin G) is preferentially bound by alpha$_2$-macroglobulin (86 %), but also by alpha$_1$-antichymotrypsin (12 %) and to minor amounts (2 %) to alpha$_1$-proteinase inhibitor [35]. About 50 % of granulocyte collagenase is bound by alpha$_2$-macroglobulin and alpha$_1$-antitrypsin each [36].

It was suggested that proteases released from granulocytes are rapidly bound to alpha$_1$-proteinase inhibitor, causing a decrease of this antiprotease [37]. Our studies showed a significant increase of alpha$_1$-proteinase inhibitor and alpha$_2$-macroglobulin activities and concentrations [28]. Calculated on a plasma protein basis, these main plasma proteinase

inhibitors remained unchanged during hemodialysis [28]. Previous studies also failed to exhibit changes of alpha$_1$-proteinase inhibitor during dialysis [12, 30]. The elimination of alpha$_2$-macroglobulin complexes with granulocyte elastase follows single exponential functions with half-lives of about 12 minutes in the circulation. Complexes of alpha$_1$-proteinase inhibitor with elastase persist longer in the circulation with a half-life of 1 hour [38]. In agreement with these results Knudsen and coworkers [39] determined predialytic E-alpha$_1$PI levels 8 hours after termination of extracorporeal circulation.

Ohlsson et al found a dissociation constant K_i below 3.5×10^{-10} for the elastase alpha$_1$-proteinase inhibitor complex [34]. Travis et al [40] found an association rate of 6.5×10^7 M^{-1} sec^{-1} for alpha$_1$-proteinase inhibitor and human neutrophil elastase. An association rate of 3.0×10^4 M^{-1} sec^{-1} was assayed following oxidation of the inhibitor induced by cigarette smoking. A complete oxidation of alpha$_1$-proteinase inhibitor would be expected to result in a 4000-fold increase in free elastase. However, the amount of free enzyme which would be expected is still vanishingly small (1.5×10^{-10} M) [40].

Granulocyte myeloperoxidase, lactoferrin, collagenase and elastase were released simultaneously following phagocytosis. The release was rapid during the first 10 minutes of phagocytosis, but after 20 minutes the amount of enzyme released increased only slightly. About 25 to 30 % of the granula content of the neutral proteases collagenase and elastase were released to the exterior of the cells, as were similar amounts of myeloperoxidase and lactoferrin [34]. On the other hand, plasma levels of the main granulocyte components underwent progressive elevation during hemodialysis [12, 22, 28-32]. Furthermore, different amounts of lactoferrin and elastase are released depending on the membrane material of the dialyzer used [41]. Specific granules degranulate before azurophil granules in the presence of immune complexes. In addition, their contents for the greater part leave the polymorphonuclear leukocytes and enter the extracellular fluid. The azurophil granules degranulate more slowly and their contents, for the greater part, degranulate into phagolysosomes [42].

Plasma levels of myeloperoxidase were unchanged comparing the effects of different dialyzer membranes [41]. Studies of Schmidt [43] demonstrated that there is not only differential release of enzymes from azurophilic and specific granules of polymorphonuclear leukocytes, but there also seems to be differential release of the neutral granulocyte proteases elastase and chymotrypsin (cathepsin G), both of which are localized in the primary granules. Elevated plasma myeloperoxidase levels in the presence of each dialyzer demonstrate that none of the membranes are truly biocompatible.

The mechanisms of degranulation of polymorphonuclear leukocytes during hemodialysis are unclear. Significant complement activation and anaphylatoxin formation were observed in patients dialyzed with cuprophan hollow-fiber membranes, whereas polymethylmethacrylate and polyacrylonitrile membranes promoted only very little complement activation [10, 22, 24, 26, 44-49]. Quite possibly that active components of C3 and C5 acting as anaphylatoxins are responsible for granulocyte activation during hemodialysis. However, recent studies from our laboratory challenge the concept that neutrophil activation during dialysis occurs solely via the activation of the complement pathway. For example, patients dialyzed with cuprophan dialyzers show significant anaphylatoxin formation but only 50 % display a remarkable elevation of plasma E-alpha$_1$PI levels [50]. Very little complement activation occurs in patients dialyzed with polymethylmethacrylate dialyzers but increased plasma levels of main granulocyte components up to 600 % were observed [22]. Membranes like polyacrylnitrile are also free of complement-activating potential. However, elevated plasma lactoferrin values (647.7 ± 203.6 ng/ml) indicate degranulation of specific granules under these conditions [41]. Plasma E-alpha$_1$PI levels were significantly higher in patients dialyzed with the polycarbonate compared with the cuprophan membrane. On the other hand, plasma C3a levels were higher in patients dialyzed with the cuprophan dialyzer [50]. Recent publications of Schaefer et al [51, 52] show that the release of granulocyte components is strictly dependent on the geometry and surface of the dialyzer. Plasma levels of E-alpha$_1$PI and lactoferrin were significantly increased during dialysis with flat sheet dialyzers as compared to hollow fiber devices. With respect to surface area, larger dialyzers tend to cause more release of granulocyte constituents as compared to dialyzers with smaller surface areas, irrespectively of the configuration of the dialyzer used. Activation of the complement system, however, did not differ with both types of configurations. The same held true for initial leukopenia [51, 52]. Tetta et al [53] studied the electric properties of membranes and showed that only cationic, but not anionic or neutral polymethylmethacrylate membranes activate plasma-free human polymorphonuclear neutrophils even in the absence of complement. Chenoweth et al [45] demonstrated that the intensity of complement activation during hemodialysis is determined by the type of dialysis membrane and whether it is new or re-used. Re-used cuprophan dialyzers displayed very little complement activation [45]. Plasma levels of granulocyte lactoferrin and myeloperoxidase, however, remained unchanged even after the 10th re-use, whereas a 50 % reduction of plasma E-alpha$_1$PI levels were observed [54]. Maggiore et al [55] recently demonstrated that the increase in C3a and C5a antigen concentrations was diminished when the patient's blood temperature was lowered to 34.2° C at the

dialyzer outlet. The leukopenia was correspondingly attenuated. In collaboration with Maggiore and coworkers we could document that plasma levels of lactoferrin, myeloperoxidase and E-alpha$_1$PI remained unaffected during cool hemodialysis (unpublished results).

Hakim and coworkers [49] suggested a close relationship between complement activation and hypersensitivity reactions to dialysis membranes. We observed a patient with hypersensitivity reaction to ethylene oxide showing plasma lactoferrin levels higher that 2,000 ng/ml during hemodialysis using ethylene oxide sterilized polysulfone dialyzer and blood lines. Plasma lactoferrin values of this patient were in the range between 300 and 400 ng/ml during dialysis with steam-sterilized or gamma-radiated cuprophan dialyzer and blood lines. Plasma E-alpha$_1$PI levels of this patient remained unaltered in the presence of different dialyzers and blood lines. We have also shown that plasma E-alpha$_1$PI levels are not influenced by the way of sterilization in patients showing no clinical signs of hypersensitivity [56].

The effect of immunosuppression (0.3 mg prednisone/kg body weight; cyclosporin A) on the plasma levels of granulocyte lactoferrin, myeloperoxidase and elastase was investigated in patients with oliguria after cadaveric renal transplantation. During hemodialysis with cuprophan, polymethylmethacrylate or polyacrylonitrile dialyzers plasma levels of both major components of the azurophilic granules were not significantly influenced by immunosuppression. Immunosuppression, however, caused significantly lower plasma lactoferrin values during hemodialysis independent of the used dialyzer [57]. Therefore, we conclude that granulocyte activation during hemodialysis does not necessarily need complement activation. Recently Betz et al [58] observed thrombocyte activation during interaction of human thrombocytes with cuprophan membranes also in the absence of complement.

Additionally, neutral proteinases of human polymorphonuclear leukocytes were followed up cytochemically in blood smears of the patients submitted to regular hemodialysis treatment. Halo formation was reduced immediately before and completely absent two hours after initiation of hemodialysis therapy with cuprophan dialyzers. Ring shaped area around each neutrophil due to erythrocyte degradation could be induced again *in vitro* at the end of hemodialysis therapy [30]. Halo formation was also reduced in 10 of 15 patients with end-stage renal failure undergoing dietary treatment [59, 60]. Halo formation of polymorphonuclear leukocytes was markedly reduced or even absent in all patients with acute renal failure [59].

Schaefer et al [61] determined plasma E-alpha$_1$PI levels in patients with acute renal failure (ARF) and sepsis. Patients with ARF displayed predialytic E-alpha$_1$PI levels of 424 ± 24 ng/ml, whereas patients on regular dialysis treatment (RDT) showed only levels of 138 ± 28 ng/ml. After 3 hours of hemodialysis with cuprophan dialyzers E-alpha$_1$PI values were comparable in both groups of patients (ARF: 668 ± 99; RDT: 679 ± 80 ng/ml). These data suggest degranulation of granulocytes in patients with acute renal failure and/or sepsis even before induction of hemodialysis. Jochum et al [62] and Duswald et al [63] observed peak values of E-alpha$_1$PI > 2,000 ng/ml in patients with septicemia after abdominal surgery.

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