Neutrophil Adhesiveness During Prostacyclin and Heparin Hemodialysis

By Philip J. Spagnuolo, Steven H. Bass, Michael C. Smith, Kowit Danviriyasup, and Michael J. Dunn

We evaluated neutrophil adhesiveness in patients undergoing chronic hemodialysis using either prostacyclin or heparin as antithrombotic agents. Patients underwent successive hemodialyses with prostacyclin (4 ng/kg/min) and heparin. There were no significant differences noted in neutrophil adhesiveness function during either dialysis: transient neutropenia developed in each case; impaired neutrophil adhesiveness to plastic developed during both dialyses; neutrophil aggregation was diminished when compared to predialysis responses during both dialyses. Furthermore, the number of circulating Fc-receptor-bearing neutrophils fell significantly during both prostacyclin and heparin hemodialysis. Our study demonstrates that substitution of prostacyclin for heparin in doses that do not cause hypotension, does not prevent neutropenia or alter the diminished neutrophil adhesiveness that occurs during heparin hemodialysis.

Hemodialysis, despite its efficacy in the management of chronic renal failure, may result in acute complications with significant morbidity. Hemorrhagic complications have been attributed to inadequately controlled heparin anticoagulation. Profound leukopenia and hypoxemia have been associated with leukocyte sequestration in the pulmonary vascular bed. Cephalophe dialyzer membranes are capable of activating the alternative pathway of complement to generate biologically active proteins such as C5a. Interaction of leukocytes with dialyzer membrane-activated plasma results in stimulation of neutrophil adherence to surfaces as well as other neutrophils and may contribute to pathologic pulmonary leukostasis, hypoxemia, and chronic sequelae including calcification and fibrosis within alveolar septae observed in hemodialysis patients.

Following the discovery of the antiplatelet properties of prostacyclin (PGI₂), an unstable metabolite of arachidonic acid metabolism, clinical studies have demonstrated the safety and efficacy of this substitute for heparin in hemodialysis. PG1₂, presumably by its stimulation of intracellular cyclic-AMP, has been shown to inhibit complement-induced increments of neutrophil adherence to nylon fiber in vitro. In the current study we compared the alteration of neutrophil adhesive function induced by hemodialysis, while comparing PGI₂ and heparin as antithrombotic agents.

Materials and Methods

Study Population and Dialysis Protocol

Twelve patients with chronic renal insufficiency receiving maintenance hemodialysis were selected for study. The patient's ages ranged from 19 to 57 yr (mean 39). The duration of hemodialysis was 27 ± 6.5 mo (mean ± SEM). The underlying cause of renal disease was chronic glomerulonephritis or interstitial nephritis. Patients with diabetes mellitus, connective tissue disorders, or those who required drugs known to affect platelet or neutrophil function were excluded from the study protocol.

The study protocol consisted of two dialyses within 14 days. Prostacyclin (sodium salt) was administered as a substitute for heparin in dialysis I. Ten of the 12 patients underwent dialysis II with heparin as the only anticoagulant. Two patients received renal transplants after the first dialysis and were excluded from the remainder of the study. The same model dialysis kidney was used for both dialyses in any individual patient. However, the type of artificial kidney varied among patients: 5 were dialyzed with cellulose acetate membranes (Cordis Dow Corporation, Miami, Fla.), and 7 were dialyzed with cuprophane fiber dialyzers (Hospal Medical Corporation, Littleton, Colo.). In the first dialysis, PGI₂ was infused intravenously by variable-speed infusion pump at 4 ng/kg/min for approximately 15 min prior to hemodialysis; during hemodialysis, PGI₂ was infused into the arterial line of the hemodialysis unit and the infusion rate was adjusted from 6 to 12 ng/kg/min based on systolic blood pressure. The total dose of PGI₂ infused during the 210 min hemodialysis varied from 37.8 to 163.8 μg with a mean dose of 97.1 ± 9.5 μg. The plasma concentration of the PGl₂ metabolite, 6-keto prostaglandin F₁α (6-KPGF₁α) averaged 1126 ± 70, 1360 ± 100, and 1210 ± 163 pg/ml after 60, 120, and 210 min of hemodialysis. Heparin was used in the second dialysis as the sole anticoagulant to maintain the activated whole blood clotting time 20–30 sec above predialysis control (i.e., initial dose of 1000–2000 U followed by an infusion of 1000–2000 U/hr during hemodialysis).

Informed consent was obtained from all patients. The Human Experimentation Committee approved the study protocol, which conformed to the principles of the Declaration of Helsinki.

Polymorphonuclear Leukocyte Adherence

Heparinized whole blood (15 U/ml) was obtained from normal healthy controls or from each patient 60 min prehemodialysis and 15, 30, and 60 min into dialysis and 15–60 min postdialysis. Neutrophils (PMN), purified as described previously, from prehemodialysis, intradialysis, and posthemodialysis blood were >98% pure. Cell yields varied, particularly at the 15-min hemodialysis.
point, because of profound neutropenia; thus, every examination could not be performed for each patient. PMN adherence to plastic was assessed by incubating 10⁶ PMN suspended in 5% patient or normal pooled plasma in 16-mm multiwell plates (Flow Labs, Hamden, Conn.) as described previously. After 60-min incubation at 37°C with or without adherence-augmenting agents, the monolayers were washed free of nonadherent cells, incubated in 30-mm xylocaine for 20 min at room temperature, scraped, and enumerated with a Coulter Counter. Adherence-augmenting factors included lipopolysaccharide from Escherichia coli 026:B6 (LPS, Difco Labs, Detroit, Mich.) and n-formyl-methionyl-leucyl-phenylalanine (FMLP, Sigma Chemical Co., St. Louis, Mo.). In some experiments, we assayed whole blood PMN adherence to nylon using 50 mg nylon fiber columns (Fenwal, Deerfield, Ill.) as described previously.

**PMN Aggregation**

PMN aggregation was examined using a platelet aggregometer/recorder system (Chronolog Model 300, Chronolog Corp., Havertown, Pa.). PMN from healthy control subjects or hemodialyzed patients (400 µl of a suspension at 10⁷/ml) were added to a cuvette containing a Teflon stir bar. The cells were stirred for 2 min to warm the suspension to 37°C; cytochalasin-B (Sigma Chemical Co.) 5.0 µg/ml was added for an additional 2 min followed by an aggregation stimulus, 10 µM FMLP. The change in light transmission with time was recorded.

**PMN Immunoglobulin Receptors**

Receptors for the Fc portion of IgG were determined by a modification of the method of Klemper and Gallin. Human red blood cells from normal volunteers were prepared by dextran sedimentation and washed three times in Hank's balanced salt solution (HBSS, K.C. Biologicals, Lenexa, Kans.) containing 0.2% bovine serum albumin (BSA, Sigma Chemical Co.). EAs (sensitized red blood cells) were prepared by incubation of equal volumes of 5% v/v suspension of red blood cells with a 1:1000 dilution of rabbit anti-human red blood cell IgG (Cappel Laboratories, Cochranville, Pa.) for 30 min at 37°C. Excess antibody was removed by washing the EAs twice with HBSS-BSA at 4°C. Control or patient PMN, suspended in HBSS-BSA (2%) at 10⁷/ml, were added to an equal volume of EAs in duplicate 6 x 50 mm glass tubes and centrifuged at 150 g at 4°C for 10 min. The pellet was incubated at 4°C for 60 min before rosettes were counted. After gentle resuspension, the percent of PMN bearing at least 3 rosetted red cells was estimated by light microscopy.

**Radioimmunoassay Determinations of 6-KPGF₁α**

Blood samples were collected into tubes containing EDTA and meclofenamate, kept on ice, and centrifuged within 15 min of sampling. Unextracted plasma was assayed for 6-KPGF₁α utilizing ¹²⁵I-6-KPGF₁α as the radioligand. Antiserum to purified 6-KPGF₁α, conjugated to keyhole limpet hemocyanin, was raised in a rabbit. The antiserum demonstrated 15% cross-reactivity with PGE₁α, 2% with PGE₂, 1.7% with PGF₂α, and less than 0.5% with PGE₃, 6,15-diketo-PGF₁α, and TXB₂. Fifty picograms of 6-KPGF₁α were required to displace 50% of the iodinated radioligand.

**RESULTS**

**Effect of Prostacyclin on Dialysis Neutropenia**

Infusion of PGI₂ did not prevent the development of neutropenia, which was maximal at 15 min in both heparin and PGI₂ dialyses (Fig. 1). In fact, neutropenia persisted to a greater degree 30 min into PGI₂ dialysis, although this difference did not reach statistical significance. Postdialysis neutrophil counts were significantly greater than predialysis counts (PGI₂ 3644 ± 618/µl, heparin 3432 ± 334/µl) in both PGI₂ and heparin dialysis (165% ± 18% and 162% ± 29% of predialysis count, respectively). The degree of neutropenia was not affected significantly by the type of dialyzer membrane used. Hypoxemia, as measured by arterial blood gas determination, was not observed during either PGI₂ or heparin hemodialysis.

**PMN Adherence During PGI₂ Hemodialysis**

Baseline adherence to plastic was 51 ± 1 x 10⁵ cells/well in 17 healthy controls. FMLP 10 µM and LPS 20 µg/ml increased adherence to plastic in controls to 210 ± 13 x 10⁵ and 143 ± 10³ cells/well, respectively. Basal adherence in patients, prehemodialysis, was greater than normal controls (p < 0.01, Student’s t test), but the stimulation of adherence by FMLP or LPS was similar to controls (Fig 2A). Basal adherence fell slightly but not significantly during PGI₂ and heparin hemodialysis. In addition, there was a reduction of responsiveness to stimulation by LPS and FMLP that occurred during both dialyses (Fig. 2B). Moreover, impaired stimulation of adherence in PMN obtained 15 min into hemodialysis appeared to be a cell-directed defect, i.e., plasma taken at 15 min into PGI₂ or heparin dialysis did not contain any factors that inhibited basal or stimulated adhesiveness of control cells (data not shown). Furthermore, PMN
adherence of patient neutrophils remained altered whether the cells were suspended in autologous plasma or pooled normal plasma. Posthemodialysis, PMN regained the profile of prehemodialysis PMN demonstrating normal adherence responses to both LPS and FMLP (Fig. 2C).

Since the half-life of infused PGI₂ is only 3–5 min²⁴ and the PMN purification procedure required several hours, we searched for a transient effect of PGI₂ on adherence that may have been inapparent using purified PMN populations. We examined whole blood, within 10 min of venipuncture, using the nylon fiber adherence assay (Table 1). In three patients, PMN obtained 15 min into PGI₂ dialysis were hyperadherent compared to predialysis value. Similar changes were observed in one patient during heparin dialysis [predialysis, 17% ± 2% adherence, dialysis (15 min), 69% ± 3%] in accord with previous observations in the literature.²⁵

PMN Aggregation During PGI₂ Hemodialysis

PMN aggregated significantly better, predialysis, in response to 10 μM FMLP compared to normal controls (Fig. 3) 1 min following stimulus addition. The difference between control and hemodialysis PMN aggregation in the first minute was no longer significant 2 and 5 min after stimulus addition (data not shown). Fifteen minutes after initiation of dialysis, aggregation responses at 1, 2, and 5 min after stimulus addition were diminished when compared to predialysis values for both PGI₂ (p = 0.07) and heparin hemodialysis (p < 0.025). PMN obtained during heparin but not PGI₂ hemodialysis showed diminished aggregation responses when compared to normal controls (p < 0.025, Student’s t test). Neutrophil aggregation response postdialysis did not differ from each other or from predialysis levels.

Alteration of PMN Receptors for IgG During Hemodialysis

Predialysis PMN demonstrated similar percentages of rosette-forming cells when compared to normal
controls, 88% ± 1%, n = 10 (Table 2). During PG12 and heparin dialysis (15 min), circulating rosette-forming PMN significantly decreased from baseline values, 30% and 20%, respectively (not significantly different from each other). The percentage of rosette-forming PMN increased postdialysis and approached predialysis values. The results were similar regardless of the concentration of antibody employed to sensitize red cells (data not shown).

**DISCUSSION**

Our study confirms the observation that profound neutropenia occurs within 15 min of the initiation of hemodialysis in patients with chronic renal failure. Furthermore, our study demonstrates that at the nadir of the leukocyte count, circulating PMN have decreased responses to agents that increase adherence to plastic surfaces and to one another concomitant with a decreased proportion of circulating Fc-receptor-bearing PMN. More importantly, substitution of PG12 for heparin does not prevent leukopenia or alter the diminished neutrophil adhesiveness that occurs during heparin hemodialysis.

Prostacyclin is a potent inhibitor of platelet aggregation and PMN adhesiveness to nylon fibers and endothelial cells in vitro. Prostacyclin also suppresses, in PMN, adherence-augmenting effects of complement-induced chemotactic factors. These in vitro observations suggested that PG12 may be useful in limiting extensive platelet and PMN aggregation, which are known to occur during heparin hemodialysis. Significant inhibition of platelet aggregation measured in vitro has been observed during PG12 hemodialysis when compared to heparin hemodialysis. However, our study demonstrates that in vivo administration of PG12 during hemodialysis did not limit potentially pathologic PMN adhesive phenomena that were predicted by previous in vitro studies.

The lack of effect of PG12 on neutrophil function in our study may reflect the low achievable serum concentrations of PG12 or rapid degradation to less active metabolites. In vitro inhibition of PMN adherence has been demonstrated at 5–100 ng/ml PG12. Peak serum concentrations of PG12 are unknown, but peak concentrations of the metabolite, 6-keto-PGF1α, ranged from 1.0 to 1.4 ng/ml during PG12 dialysis. Although the levels of 6-keto-PGF1α probably underestimate and lag behind peak PG12 concentrations, the levels obtained in our patients were at the lower limits of previously observed in vitro inhibition of PMN adherence. Thus, our results could reflect inability to achieve levels of PG12 associated with functional suppression of PMN adhesiveness in vitro. Unfortunately, greater PG12 infusion rates were precluded because of clinical hypotension.

We also examined whole blood adherence to nylon fiber, an assay that obviates the necessity for time-consuming purification of PMN. No differences were noted between whole blood adherence during PG12 or heparin hemodialysis. In both cases, PMN obtained 15 min into dialysis demonstrated increased adherence to nylon, suggesting we had not overlooked a transient, reversible effect of PG12 on adhesiveness. Interestingly, our data demonstrate an apparent paradox: while whole blood adherence is increased 15 min after initiation of hemodialysis, adherence of purified PMN to plastic is suppressed. MacGregor et al. also demonstrated enhanced PMN adherence to nylon during the first 15 min of hemodialysis, in contrast, Klempner and Gallin showed a decrease in adherence of purified neutrophils to plastic during dialysis. Although the patients studied were different (MacGregor’s patients had chronic renal failure, whereas Klempner’s patients were normal physiologically), it seems likely that the differences in assay systems may explain the discrepant results. In fact, exposure to nylon fiber may cause release of PMN lysosomal contents and result in further activation of cells possibly explaining the enhanced adhesiveness observed using the nylon fiber assay.

Our data also demonstrate a loss of Fc-receptor-bearing PMN from the circulation during PG12 and heparin hemodialysis. Similar observations were made by Klempner and Gallin in a group of diazyed schizophrenic volunteers. These investigators have suggested that altered circulating neutrophil function, e.g., abnormal adhesiveness and aggregation, during hemodialysis results from a selective loss due to margination of functionally superior Fc-receptor-bearing neutrophils. Our observations tend to support their hypothesis; however, our studies demonstrate a dissociation between Fc receptor activity and PMN aggregation responses during PG12 hemodialysis. Although adherence to plastic and the proportion of Fc-receptor-bearing PMN decreased during both PG12 and heparin hemodialysis, PMN aggregation during heparin HD was significantly reduced, while PMN obtained during

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<th>Table 2. PMN Fc Receptor Alterations During Hemodialysis*</th>
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<td>Type of Dialysis</td>
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*Results are expressed as mean ± SEM of duplicate determinations for each patient.

†Percent Fc-positive PMN.

‡Differs from predialysis by paired sample t test, p < 0.025.
PGI₂ hemodialysis aggregated less well than predialysis PMN, but normally in comparison to control PMN.

Although this might suggest preservation of selective PMN function during PGI₂ hemodialysis, the similar degrees of intradialysis neutropenia and PMN adhesion abnormalities would argue against such a possibility. More likely, it appears that there may be a dissociation of Fc receptor activity and PMN aggregation and that Fc-receptor-negative cells isolated by rosetting techniques still may be capable of aggregating, although less well than Fc receptor positive cells (Fain M, Bass SN, Spagnuolo PJ: Dissociation of neutrophil aggregation and Fc receptor expression. Clin Res 29:718, 1981, abstr).

Our study demonstrates that the normal physiologic response to heparin hemodialysis in patients with chronic renal failure results in neutrophil aggregation and sequestration manifested by neutropenia and selective margination of functionally more adhesive Fc-receptor-bearing PMN. The PMN remaining within the vascular compartment demonstrated diminished adherence and aggregation and a decreased proportion of Fc-receptor-bearing cells. Although previous in vitro experiments have shown that prostacyclin was antiaggregatory for PMN, in vivo administration of PGI₂ at doses used in this study did not alter the pathophysiologic PMN response to hemodialysis. Attempts to prevent uncontrolled leukocyte adherence and aggregation during hemodialysis and their potential long-term pulmonary sequelae should probably be directed at modifying dialyzer membranes, since higher infusion rates of PGI₂ may result in significant hypotension. Recent evidence suggests that dialyzer membranes composed of polyacrylonitrile or polycarbonate result in less complement activation and neutropenia than cellophane or cuprophane dialyzers. 30 Whether polycrylonitrile dialyzers will alter hemodialysis-induced leukoaggregation or prevent pathologic sequelae in patients will require study.

REFERENCES


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