TRANSLATIONAL PHYSIOLOGY

Nocturnal hemodialysis is associated with restoration of impaired endothelial progenitor cell biology in end-stage renal disease

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Submitted 31 March 2005; accepted in final form 24 May 2005

Chan, Christopher T., Shu Hong Li, and Subodh Verma. Nocturnal hemodialysis is associated with restoration of impaired endothelial progenitor cell biology in end-stage renal disease. Am J Physiol Renal Physiol 289: F679–F684, 2005.—Cardiovascular disease is the principal cause of death in end-stage renal disease (ESRD) patients. Endothelial progenitor cells (EPCs) play a critical role in vascular repair, and improving EPC biology represents a novel therapeutic target. Three groups of age- and gender-matched patients were studied: 1) 10 healthy control, 2) 12 conventional hemodialysis (CHD) patients, and 3) 10 nocturnal hemodialysis (NHD) patients. EPC number and migratory function were assessed. Left ventricular mass index (LVMI) was derived, and correlations between EPC biology, uremic clearance, and LVMI were made. Compared with controls, EPC number and function were markedly impaired in CHD patients [(3.48 ± 1.2 vs. 0.85 ± 0.20%/50,000 cells, P < 0.05) and (18.8 ± 2.64 vs. 3.75 ± 0.34 cells/high-power field, P < 0.05), respectively]. In contrast, EPC number and function were normal in NHD patients [(3.48 ± 1.17 vs. 3.83 ± 0.77%/50,000 cells) and (18.8 ± 2.6 vs. 22.2 ± 2.4 cells/high-power field), respectively]. Among ESRD patients, EPC number and function inversely correlated with predialysis urea concentration (r = −0.40; r = −0.57), LVMI (r = −0.41; −0.46) and systolic BP (r = −0.58; r = −0.44). We demonstrate that NHD is associated with restoration of abnormal EPC biology in ESRD. Given the increasing importance of EPCs in the repair and restoration of cardiovascular function, these data have important clinical implications for vascular risk in ESRD patients.

Nocturnal home hemodialysis (NHD), which provides 8–10 h of renal replacement therapy during sleep, five to six nights per week, is a potentially beneficial mode of dialysis for the ESRD population. The Toronto NHD experience has documented significant cardiovascular improvements in patients following conversion (from CHD) to NHD, including improved blood pressure (BP) control (30), reduction in antihypertensive drug requirements (7), improvement in endothelial-dependent dilation (9), regression of LV hypertrophy (7), recovery of impaired LV systolic function (6), and restoration of nocturnal cardiac autonomic balance (8). However, a critical question that remains unanswered is whether hemodialysis improves abnormal EPC biology in ESRD, and more specifically, whether this effect is modulated to a greater extent by NHD compared with conventional hemodialysis (CHD).

We conducted a cross-sectional study to test the following hypotheses: 1) augmentation of uremic clearance by NHD is associated with improved EPC number and function to a greater extent than CHD; and 2) EPC number and function are directly related to LV geometry, BP, and uremic control in ESRD patients.

METHODS

This protocol was approved by the Research Ethics Board of the Toronto General Hospital, University Health Network, Toronto, Canada. The following three groups of age- and gender-matched patients were studied: 1) healthy control (n = 10); 2) ESRD patients on CHD (n = 12); and 3) ESRD patients on NHD (n = 10). The NHD group represented patients who had been on this mode of dialysis for a minimum duration of 1 yr. None of the patients had any acute illness dysfunction is a key determinant of adverse vascular outcomes in ESRD (16, 27). Bone marrow-derived endothelial progenitor cells (EPCs) play a critical role in endothelial maintenance (29). In addition to facilitating angiogenesis, EPCs can transdifferentiate into cardiomyocytes with the potential to the repair and regenerate ischemic myocardium (5, 43). Reduced EPC number and function correlate inversely with vascular risk factors (20), and endothelial dysfunction has been observed in patients with renal failure (39). An emerging body of data suggests that strategies aimed at improving EPC survival and function improve cardiovascular function, in both experimental and clinical settings (4, 37). Indeed, EPC transplantation was effective in preserving left ventricular (LV) geometry and systolic function in human models of myocardial ischemia (23).

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or symptomatic cardiovascular disease (including congestive heart failure and acute coronary syndrome). Written informed consent was obtained from each patient.

HD patients received hemodialysis at home for 6–8 h, five to six nights per week. Vascular access was achieved through either a long-term internal jugular catheter (Udall Catheter, Cook Critical Care, Bloomington, IN) or an arteriovenous fistula. A dialysate flow rate of 350 ml/min, a blood flow rate of 200–300 ml/min, and Polyflux polyamide dialyzers (Gambro, Lund, Sweden) were used for each treatment. CHD patients received hemodialysis for 4 h three times per week via similar vascular access. A blood flow rate of 400 ml/min, a dialysate flow rate of 500–750 ml/min, and F80 polysulfone dialyzers (Fresenius Medical Care, Lexington, MA) were used. Unfractionated heparin was used for anticoagulation on CHD and NHD.

In both ESRD cohorts, clinical assessment, including weight, height, BP, and routine dialysis-related biochemical analyses, was performed. Dialysis dose per treatment was estimated by equilibrated Kt/V (eKt/V) as described by Daugirdas and colleagues (11), where eKt/V = spKt/V - 0.6(spKt/V)/t + 0.03 (spKt/V = single pool Kt/V, K = dialysate clearance, and t = dialysis time, and V = urea distribution volume). Single-pool Kt/V was determined using the blood urea reduction ratio (22). Seated BP in each patient after 5 min of rest was measured by the principal investigator during a clinic visit. All BP measurements were obtained with the same calibrated sphygmomanometer. Echocardiographic data were obtained for each ESRD patient and interpreted blindly. Left ventricular mass was calculated from two-dimensional echocardiographic images according to the formula of Devereux and Reichek (13). Left ventricular mass index (LVMI) was derived by correcting the left ventricular mass for a body surface area of 1 m². Left ventricular fractional shortening (FS) was calculated as FS = (LVED – LVES)/LVED, where LVED is left ventricular internal end-diastolic dimension and LVES is left ventricular internal end-systolic dimension.

Prescribed cardiovascular medications were documented. These included diuretics, β-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, digitals, calcium channel blockers, and vasodilators. The dose of erythropoietin (EPO) prescribed was also documented, given the well-established effects of EPO on EPC number and function (19).

EPC number and function were assessed as described below. Blood samples were obtained at midweek and predialysis for CHD patients. To minimize circadian variation and replicate steady-state CHD and NHD conditions, blood samples were drawn at the same time of day for NHD patients (a minimum of 4 h after the end of a regular NHD session). Vasoactive medications were withheld for a day until after the blood samples were obtained. All samples were processed within 2 h after collection.

EPC isolation. EPCs were isolated by enriched medium isolation as described previously (41). Briefly, peripheral venous blood was taken from ESRD patients and normal control subjects, the mononuclear cell fraction was isolated by Ficoll-Paque density gradient (Becton Dickinson) centrifugation and washed three times with PBS (Sigma), and cells were plated at a density of 10⁶ mononuclear cells/cm² on fibronectin-coated culture slides (Becton Dickinson) in endothelial cell (EC) basal medium-2 (EBM-2; Clonetics) supplemented with endothelial growth medium SingleQuots and 20% fetal bovine serum.

EPC phenotyping. Serum samples from ESRD patients and normal controls were collected in heparinized tubes. Blood (2-3 ml) was lysed twice with cell lysis buffer (154.4 mM ammonium chloride, 11.9 mM sodium bicarbonate, 26 mM EDTA). After cell lysis, one million cells were taken for antibody staining. Endothelial identity was confirmed with antibody recognizing FITC-conjugated human vascular endothelial (VE)-cadherin (Seretec) and biotin-conjugated monoclonal antibody against PE-conjugated CD34 (Pharmingen). All antibody incubation was carried out for 30 min at 4°C in the dark. Isotype-identical served as control (Becton Dickinson). Cells were analyzed using a Beckman Coulter EPICS XL flow cytometer with EXPO32 ADC software. The fluorescence intensity of 50,000 cells for each sample was quantified.

EPC migration assay. EPC migratory response to vascular endothelial growth factor (VEGF) was assessed. After 4 days in culture, nonadherent cells were removed by thorough washing with PBS, adherent EPCs were detached using trypsin/EDTA (GIBCO), harvested by centrifugation, resuspended in 200 μl EBM, and counted, and 2 × 10⁵ EPCs were placed in the upper chamber of a Transwell chamber with a polycarbonate membrane (Corning). The chamber was placed in a 24-well culture dish containing EBM with 10% FBS and human recombinant VEGF (50 ng/ml). After 24-h incubation at 37°C, the lower side of the membrane was washed with PBS and fixed with methanol. For quantification, cell nuclei were stained with hematoxylin. Cells migrating into the lower chamber were counted manually in five random microscopic fields.

Data analysis. The primary outcome measures were differences in EPC number and migratory function among normal controls, CHD, and NHD patients. LVMI, BP, predialysis urea, and other dialysis-related biochemical parameters were also compared between CHD and NHD cohorts. Descriptive analyses are presented as means ± SE. A Mann-Whitney U-test was used for comparison of continuous variables between two groups. Analysis of variance was used for multiple comparisons of a continuous variable among three groups of subjects. Spearman’s correlation was used to investigate potential associations between variables of interest. All statistical tests were two tailed with a P value <0.05 taken to indicate significance. SPSS-10 (SPSS, Chicago, IL) was used for all statistical analyses.

RESULTS

Demographic characteristics of the three groups of patients are presented in Table 1. The groups were age matched, and none of the patients had symptomatic cardiovascular disease. The clinical data for all ESRD patients are shown in Table 2. The sessional dialysis dose (eKt/V) delivered to the NHD patients was significantly higher than the CHD cohort (1.40 ± 0.05 to 2.01 ± 0.14, P < 0.05) (Table 2). In addition, the frequency of dialysis doubled. As expected, predialysis urea, creatinine, and phosphate concentrations were lower in the NHD group due to intensive hemodialysis compared with the CHD cohort. Other dialysis-related biochemical parameters were similar between the two groups. BP was adequately

Table 1. Demographic characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal (n = 10)</th>
<th>CHD (n = 12)</th>
<th>NHD (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>41 ± 4</td>
<td>41 ± 3</td>
<td>42 ± 4</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>7:3</td>
<td>8:4</td>
<td>7:3</td>
</tr>
<tr>
<td>Etiology of ESRD</td>
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<td>HTN: 3</td>
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<tr>
<td>Congenital</td>
<td>Congenital: 3</td>
<td>Congenital: 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GN: 2</td>
<td>GN: 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DM: 3</td>
<td>DM: 1</td>
<td></td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>None</td>
<td>2.3 ± 0.4</td>
<td>0.3 ± 0.2*</td>
</tr>
<tr>
<td>Medications, per patient</td>
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<td></td>
</tr>
<tr>
<td>α-Blocker</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ARB</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>β-Blocker</td>
<td>8</td>
<td>1</td>
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</tr>
<tr>
<td>Calcium channel blocker</td>
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</tr>
<tr>
<td>Diuretics</td>
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<td></td>
</tr>
<tr>
<td>Vasodilators</td>
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</table>

Values are means ± SE. M, male; F, female; ACE inhibitor, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CHD, conventional hemodialysis; NHD, nocturnal home hemodialysis; ESRD, end-stage renal disease; DM, diabetes mellitus; GN, glomerulonephritis; HTN, hypertension. *P < 0.05 compared with CHD.
controlled in both groups; however, the requirement for vasoactive medications and EPO was higher in the CHD group. Despite the greater need for antihypertensive medications, the systolic BP was higher in the CHD group compared with NHD patients (143 ± 3 vs. 128 ± 3 mmHg, $P = 0.002$). Similarly, the hemoglobin concentration was higher in the NHD group compared with the CHD cohort (132 ± 5 vs. 115 ± 2 g/l, $P < 0.001$) despite greater use of EPO in the CHD group. The use of statins was similar between the two patient populations. Left ventricular geometry was better preserved in the NHD cohort. Both CHD and NHD patients had normal LV systolic function as assessed by LV FS.

EPC number and migratory function were markedly impaired in ESRD patients subjected to CHD [(EPC number: 3.48 ± 1.2 vs. CHD 0.86 ± 0.20%/50,000 cells, $P < 0.05$) and (EPC function: 18.8 ± 2.64 vs. CHD 3.75 ± 0.34 cells/high-power field, $P < 0.05$)] (Table 3). Importantly, these derangements in EPC biology were observed despite higher erythropoietin consumption, which is known to favorably modulate EPC survival and function. In contrast, NHD patients exhibited normal EPC number and function compared with healthy controls [(EPC number: 3.48 ± 1.17 vs. NHD 3.83 ± 0.77%/50,000 cells, $P > 0.05$) and (EPC function: 18.8 ± 2.6 vs. NHD 22.2 ± 2.4 migrated cells/high-power field, $P > 0.05$)] (Table 3). Stratification according to diabetic status did not alter the results, suggesting that uremic clearance may have a direct influence on EPC number and function.

Among ESRD patients, EPC number and function inversely correlated with predialysis urea concentration ($r = -0.40$, $P < 0.05$; $r = -0.57$, $P < 0.05$, respectively). In addition, EPC number and function inversely correlated with LVMI ($r = -0.41$, $P < 0.05$; $r = -0.46$, $P < 0.05$, respectively) and systolic BP ($r = -0.58$, $P < 0.05$; $r = -0.44$, $P < 0.05$, respectively) in ESRD patients (Figs. 1 and 2).

### DISCUSSION

Cardiovascular events are the leading cause of morbidity and mortality in ESRD patients. The etiology of cardiovascular...
pathology in ESRD is complex, involving ongoing myocardial injury compounded by volume and pressure overload, endothelial dysfunction, and progressive uremic arteriopathy (25). The malignant nature of uremic cardiovascular disease was first described by Lindner et al. (24) in the 1970s and yet, to date, the annual mortality rate of ESRD patients still remains unacceptably elevated. Hypertension and diabetes, which often accompany ESRD, only partly explain the heightened cardiovascular risk, with the increasing recognition that mechanisms directly related to kidney failure come into concert to promote cardiovascular collapse in these patients (34). Of particular concern is the fact that conventional thrice-weekly hemodialysis does not appreciably alter the natural history of ESRD with respect to adverse cardiovascular outcomes.

The endothelium is the monolayer of endothelial cells lining the lumen of all blood vessels. These cells function as a protective biocompatible barrier between all tissues and the circulating blood, functioning in both an autocrine and paracrine fashion. Endothelial function is an important barometer of overall vascular risk and correlates with established and emerging cardiovascular risk factors (16). Endothelial cell dysfunction contributes to the development of vascular complications in ESRD and may represent the common denominator of a number of interrelated inflammatory, proatherosclerotic, and prothrombotic processes that are upregulated with kidney failure (48, 49). As a result, improving endothelial function has important mechanistic and therapeutic implications in ESRD.

EPCs are bone marrow-derived stem cells that have the ability to differentiate into functional endothelial cells. EPCs circulate in the blood and appear to home preferentially to sites of vascular or tissue injury, contributing significantly to both reendothelialization and neovascularization (38). However, if EPC mobilization from the bone marrow and recruitment to sites of vascular injury are impaired, vascular homeostasis may be deviated toward endothelial dysfunction and susceptibility to atherogenesis (40). A recent study suggested that in healthy men, levels of circulating EPCs may be a surrogate biological marker for vascular function and cumulative cardiovascular risk (20). The authors propose that EPCs maintain endothelial function in mature blood vessels and that the failure of this function may lead to endothelial dysfunction and abnormal vasoreactivity. The detrimental effect on EPC number may arise from impaired mobilization or a depletion of EPC supply due to continuous endothelial damage and repair. Furthermore, the number and migratory activity of circulating EPCs are decreased in patients with risk factors for coronary artery disease, suggesting that a lack of EPCs may contribute to impaired vascularization within these patients (40). Risk reduction therapies known to reduce cardiovascular events, such as the use of statins (42), can enhance the mobilization of EPCs from the bone marrow and increase the number within the circulation. Of current interest is the notion that EPCs may transdifferentiate into cardiomyocytes and integrate with the adjacent cells via gap junctions, in an effort to repair and regenerate an ischemic myocardium (21).

Given the above preamble, it is tempting to speculate that impaired EPC survival and function may be mechanistically linked to cardiovascular disease in ESRD. Indeed, a recent paper demonstrated impaired number and function of EPCs in patients with ESRD (10). However, a critical question that remains unanswered is whether hemodialysis, either CHD or NHD, can modify derangements in EPC biology.

The present study is the first to address this very question and made two novel observations. First, impaired EPC number and function in ESRD are not corrected with thrice-weekly CHD. Indeed, compared with control patients, ESRD patients on CHD exhibited an approximately fourfold reduction in EPC...
number and function. Second, augmentation of uremic clearance using NHD is associated with restored EPC number and migratory function to that observed in the normal control population. Furthermore, in ESRD patients, EPC number was related to predialysis urea concentration, systolic BP, and LVMI. In this fashion, the present study identifies a novel potential mechanistic link between uremic toxin burden, EPC number and function, and important surrogate outcomes, such as LVMI, and underscores the possible importance of aggressive uremic clearance, using NHD, for EPC survival, and function. Furthermore, we speculate that these results may explain, in part, the accelerated cardiovascular phenotype seen in ESRD patients treated with conventional renal replacement therapies.

It is plausible that repetitive ongoing cardiovascular injury in addition to the uremic milieu seen in patients undergoing CHD may exhaust the presumed finite supply of EPC and impair EPC functions (10, 33). Indeed, we and others (10, 14) have confirmed that CHD patients, despite receiving EPO (19) and other agents such as statins (42) and angiotensin-converting enzyme inhibitors (36), exhibited decreased EPC numbers and functions compared with non-ESRD cohorts. The observation that NHD (vs. CHD) exhibited improved EPC number and function in ESRD is potentially explained by sustained improvements in uremic clearance associated with NHD. In addition to benefits for EPC biology, NHD affords a number of other cardiovascular benefits in ESRD, which suggest that NHD may promote better cardiovascular outcomes (than CHD). Regression of LV hypertrophy (7), improvement in BP control, and amelioration of endothelial function (9) highlight the cardiovascular restorative potential of NHD. In the present study, we observed significant inverse correlations between EPC number and functions and predialysis urea, systolic BP, and LVMI, which, in turn, have all been shown to be highly predictive of cardiovascular death in ESRD (3, 17, 26). Our group has demonstrated that NHD lowers BP by selectively decreasing total peripheral resistance, augmenting brachial artery reactivity, and lowering plasma norepinephrine (9). We have also proposed that the improvement in vascular resistance contributes to the regression of LV hypertrophy in ESRD patients (7). Although it is possible that improvement in BP control by NHD may independently lead to amelioration of LV geometry and restoration of endothelial function, it is tempting to speculate that NHD may favorably affect a fundamental process of endothelial repair via normalizing EPC number and function, thereby resulting in improved cardiovascular outcomes. The interactions between lower systolic BP, improved vascular resistance, and restored EPC biology are largely unknown and require further studies.

Although the mechanisms of reduced EPC survival in ESRD remain unknown, it is possible that increased inflammatory cytokines and oxidative stress observed in ESRD may be the culprits. Indeed, inflammatory biomarkers such as C-reactive protein have been shown to reduce EPC number and function (41). Inflammation and CRP are predictive of cardiovascular death in ESRD and are associated with uremia clearance (32, 45), and hence it is possible that reduced EPC survival in ESRD, and its correction by NHD, may be linked to a greater reduction in the inflammatory milieu. Further studies are required to examine the impact of intensive hemodialysis on inflammation and EPC biology. Another candidate pathway involves the decrease bioavailability of nitric oxide (NO) in ESRD. It is known that inhibitors of NO synthase such as asymmetric dimethylarginine (ADMA) are increased in ESRD and that deficient NO bioavailability is associated with endothelial dysfunction, inflammation, and LV hypertrophy in the dialysis population (12, 46, 47). Recent evidence suggests that NO synthase is critical in the mobilization of EPC into the peripheral circulation (1). Hence, it is possible that NHD by virtue of improved NO bioavailability augments EPC mobilization. Finally, the influence of uremia on bone marrow biology merits an important consideration. It is generally accepted that uremia attenuates hematopoiesis (18) and leukocyte functions (31). It is interesting to note that despite a lesser requirement of EPO in the NHD patients, their hemoglobin concentrations were higher than that of the CHD group, which might suggest that enhanced uremia clearance with NHD may favorably impact hematopoiesis and EPO responsiveness (35, 44).

In summary, we highlight the concept that augmented uremia clearance using NHD is associated with restoration of EPC number and migratory function in ESRD patients. Patients on conventional renal replacement regimens, despite high doses of EPO, continue to exhibit a four- to fivefold reduction in EPC number and function. The present study represents the first attempt to study the impact of NHD on EPC biology. Additional experiments using other EPC functional assays (e.g., endothelial tube formation) and serum-mixing strategies are required to improve our basic understanding of the influence of uremia on bone marrow-derived stem cells. The true impact of extracorporeal circulation and diabetic status on EPC biology in ESRD also requires further study. Although our results are limited by its observational nature, given the important role of EPCs in vascular repair and regeneration, we believe our work adds support to the growing cardiovascular benefits of NHD and provides a rationale for further testing the impact of NHD on EPC biology and its effects on cardiovascular outcomes in ESRD patients.

GRANTS

This study was supported by the Heart and Stroke Foundation of Canada (Operating Grant NA 5571).

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