Original Article

Circulating Progenitor Cells after Cold Pressor Test in Hypertensive and Uremic Patients

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Endothelium was initially considered an inert lining of the blood vessels. Recently, it was suggested that damaged cells are continuously replaced by novel cells, hematopoietic stem cells (HSCs), which are directly mobilized by the bone marrow and then transformed into endothelial progenitor cells (EPCs). Initial triggers of vessel remodeling are physical forces such as blood pressure and fluid shear stress. We investigated whether or not a stress stimulus on vessels applied by a cold pressor test (CPT) would stimulate the mobilization of progenitor cells. Twenty-two healthy subjects, 20 patients with essential hypertension, and 18 with chronic kidney disease (CKD) underwent CPT by dipping their hands in icy water for 4 min. Immediately before and after 4 and 60 min, we quantified HSCs and EPCs identified by flow cytometry. We measured also adhesion soluble molecules (sICAM-1, sVCAM-1, and sE-selectin) as markers of endothelial activation. In healthy and hypertensive subjects, but not in CKD subjects, the number of HSCs was elevated as a direct response to CPT stress. Levels of EPCs and adhesion soluble molecules increased significantly, but to a different extent in every group. In CKD patients, the number of EPCs did not return to basal levels either after 60 min. Levels of adhesion soluble molecules directly correlated with the number of progenitor cells in hypertensive and healthy subjects. CPT caused an increase in adhesion soluble molecules. Discrepancies in the numbers of HSCs and EPCs in CKD patients could suggest a specific impairment in blood vessel remodeling correlated with recognized endothelial dysfunction. (*Hypertens Res* 2008; 31: 717–724)

***Key Words*: endothelial progenitor cells, blood pressure, cold pressor test, hypertension, uremia**

Introduction

The endothelium, the first barrier between the intravascular compartment and the interstitium, is particularly sensitive to mechanical stimuli, such as blood pressure and shear stress (*1*). In response to these physical insults, the endothelium releases substances that regulate hemodynamics and angiogenesis (*2*). In contrast to the classical view of the endothelium as an inert lining for blood vessels, recent reports suggest that there is a finely tuned, continuous turnover of endothelial cells, in which damaged cells are shed in the circulation and replaced by new endotheliocytes. These cells can originate in the endothelial cells adjacent to the lesions, or in cells directly mobilized from the bone marrow, which display both a hematopoietic and an endothelial lineage differentiation potential. When differentiating into the endothelial lineage, these cells are transformed into so-called endothelial progenitor cells (EPCs) (*3*). Stimuli altering cardiovascular integrity, such as acute trauma, ischemia, or shear stress, are known to determine a rapid transitory increase in progenitor cells (*4*). In the literature, attention has been focused on stimuli mobilizing progenitor cells in pathological conditions. It is now widely accepted that the initial trigger of blood vessel remodeling may be a physical force, such as fluid shear stress (*5*). With this concept as its starting point, the present study

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Table 1. Baseline Characteristics of CKD, Hypertensive Patients and Healthy Control Subjects

Variables	Healthy subjects (n=22)	Hypertensive (n=20)	CKD (n=18)
Age, years	50.8±10.5	48.8±12.6	51.0±13.7
Sex (M/F)	10/12	10/10	10/8
Body mass index, kg/m^2	24.6±4.80	24.3±8.62	20.5±7.6
Hemoglobin, g/dL	14.4±2.44	14.3±2.12	13.4±2.6
Hematocrit	40.3±4.94	41.3±4.12	35.4±3.12
Cholesterol, mg/dL	203.5±60.2	204.8±30.5	206.1±40.3
HDL cholesterol, mmol/L	1.23±0.02	1.25±0.06	1.24±0.07
HDL-cholesterol ratio	0.30±0.03	0.29±0.03	0.28±0.03
Triglycerides, mmol/L	0.84±0.08	0.85±0.08	0.82±0.11
Glucose, mmol/L	4.53±0.12	4.48±0.12	4.45±0.16
Glomerular filtration rate, mL/min	120.0±9.7	98.0±7.6	40.0±9.3

CKD, chronic kidney disease; M, male; F, female; HDL, high-density lipoprotein.

aimed to investigate whether or not a sudden physiologic stimulus on vascular vessels, applied by the cold pressor test (CPT), could stimulate the mobilization of EPCs from the bone marrow, to test the hypothesis that endothelial cells damaged by hemodynamic stress are thus replaced. A search was also made for vascular remodeling processes, through the analysis of the concentrations of soluble adhesion molecules (ICAM, VCAM, E-selectin), which are biochemical markers of endothelial activation (*6*, *7*). The study was conducted on patients with essential hypertension and chronic kidney disease (CKD), who were assumed to have had altered repair mechanisms. The control group consisted of age matched volunteers.

Methods

A total of 60 subjects (30 women; 30 men) enrolled in the study were divided into three groups (Table 1): 1) 20 hypertensive patients (mean age, 48.80±12.60 years); 2) 18 CKD patients (mean age, 51.00±13.70 years); 3) 22 healthy subjects (mean age 50.80±10.50 years). The body mass index (BMI) for each patient group was 24.30±8.62, 20.50±7.60, 24.60±4.80 kg/m^2, respectively. Exclusion criteria included alcohol consumption, cigarette smoking or drug abuse; suspected or confirmed secondary hypertension; heart or liver disease; thyroid disease; postural hypotension; diabetes mellitus; and/or treatment with nonsteroid anti-inflammatory drugs. The causes of CKD were: glomerulonephritis (n=5); chronic interstitial nephropathy (n=6); polycystic kidney disease (n=4), and chronic pyelonephritis (n=3). Patients considered hypertensive were those with blood pressure >160/90 mmHg in at least four sets of readings taken at 1-week intervals, along with a glomerular filtration rate (GFR) of 98.00±7.60 mL/min. These patients were treated with diuretics, calcium channels blockers, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin II type 1 (AT1) receptor blockers. In hypertensive patients, other diseases were ruled out through medical history, clinical examination, blood chemistry, urinalysis, ECG, echocardiography, and renal and carotid ultrasound evaluation. CKD patients, who had a mean residual GFR of 40.00±9.30 mL/min, were given the same antihypertensive drugs as the previous group; they also took vitamin D analogues and calcium-containing phosphate binders. Neither erythropoietin nor their analogues were administered during the 2 weeks prior to the experimental protocol. The blood pressure of the healthy volunteers was less than 130/80 mmHg, and GFR was 120.00±9.70 mL/min; in these control subjects, atherosclerotic disease, hypertension, diabetes, and hypercholesterolemia were ruled out on the basis of their clinical history and findings at the clinical examination. None of the control subjects were smokers or on drugs, and all of them led sedentary lives. Patients under antihypertensive therapy discontinued their treatment for 2 weeks before the start of the study. The local ethics committee approved the study, and fully informed consent to take part in the study was obtained in writing from all subjects.

Study Design

CPT was performed as above and in accordance with Hines and Brown, who introduced the test (*7*, *8*). At 7 AM, after an overnight fast, an indwelling catheter was inserted into the antecubital vein to draw blood, the first samples of which were taken at 8 AM Blood pressure was measured after the subjects had rested supine for 60 min in a room maintained at 25 to 30°C. Using an automated measurement and recording device (Sentry Equipment, Oconomowoc, USA), systolic and diastolic blood pressures were monitored throughout the experimental period. All blood samples, taken at the same time of day to minimize any circadian effect, were processed within 2 h after collection. CPT was performed 5 min after basal measurements (T_0) were obtained. The patients' hands were immersed to just above the wrists in cold water (3–5°C) for 4 min. Serum samples were taken at T_0, at 4 min (immediately after hands were removed from the water; T_4) and at 60 min (T_{60}) to measure serum sICAM-1 (ICAM-1 Predicta kit,

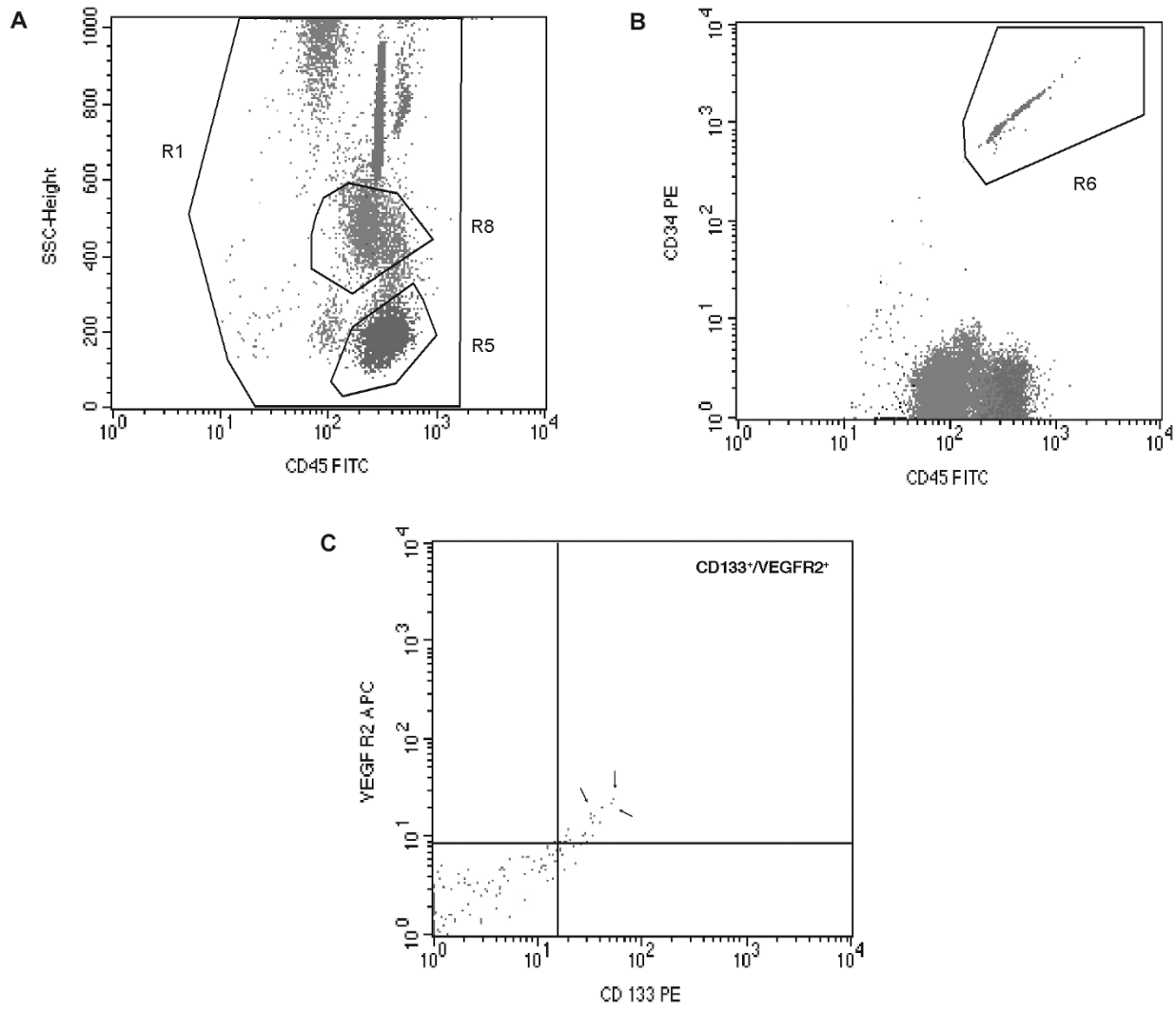

Fig. 1. *Circulating endothelial progenitor cells were identified by flow cytometry with the expression of cell surface antigens, such as CD34, CD133, and VEG-R2. Representative flow cytometry analysis of a blood sample from a CKD patient after CPT. A: FITC fluorescence in a dot plot of CD45-FITC vs. side scatter (SSC) to exclude debris and ensure that all leukocyte populations and microbeads were included. B: The R6 region was created in an ungated dot plot of PE vs. FITC fluorescence to include the microbeads. C: Dual expression of CD133$^+$/VEGFR2$^+$ cells defined as EPCs (indicated by arrows).*

Genzyme, Cambridge, USA), sE-selectin and sVCAM-1 (Bender Med System, Genzyme) and to quantify EPCs. Plasma norepinephrine and epinephrine were also measured at the same times (*7*).

Quantification of EPCs

In the present study, fresh blood cytofluorimetry was used to quantify EPCs. Cytofluorimetry is considered the gold standard for the quantitative enumeration of EPC: it is sensitive, accurate and reproducible (*9*). All peripheral blood specimens were collected and stored in 0.34 mol/L K_3EDTA anticoagulant, and all processing was completed within 2 h. Peripheral blood progenitor cells were analyzed for the expression of cell surface antigens with direct three-color analysis using fluorescein isothiocyanate (FITC)–conjugated, phycoerythrin (PE)-conjugated, and allophycocyanin (APC)-conjugated monoclonal antibodies (mAbs) by flow cytometry analysis (FACSCalibur; BD, Franklin Lakes, USA), as reported elsewhere (*10*, *11*). Briefly, before staining with specific monoclonal antibodies, cells were treated with fetal calf serum for 10 min, after which samples were washed with a buffer containing phosphate-buffered saline and 0.5% bovine albumin. Then, 50 µL of peripheral blood was incubated with 10 µL of FITC-conjugated anti–human CD45 mAb (BD), with 10 µL PE-conjugated anti–human CD34 mAb (BD), or with 5 µL of PE-conjugated anti–human CD133 mAb (Miltenyi Biotec, Bergisch Gladbach, Germany), and 10 µL of APC-conjugated

anti–human KDR mAb (R&D Systems, Minneapolis, USA) followed by incubation at room temperature for 15 min in the dark. 7-Amino-actinomycin D (7-AAD, VIAPROBE, BD Pharmingen, Heidelburg, Germany) was added to determine viable cells and to exclude dead cells. To avoid cell loss, no wash was performed. Flow cytometric acquisition and analysis were performed using FACS VANTAGE (BD); the cytometer was equipped with a 488 nm argon laser and a 635 nm red-diode laser. The threshold was set on FITC fluorescence in a dot plot of CD45-FITC *vs*. side scatter (SSC) to exclude debris and ensure that all leukocyte populations and microbeads were included (Fig. 1A). The R6 region was created in an ungated dot plot of PE *vs*. FITC fluorescence to include the microbeads (Fig. 1B). Acquisition was stopped when a minimum of 5,000 events were present in this region. Gating strategies and sample analysis allowed the measurement of the absolute count of $CD34^+$ and dual expression of $CD133^+/VEGFR2^+$ cells (Fig. 1C). $CD34^+$ cells were defined as hematopoietic stem cells (HSCs), whereas $CD133^+$ $VEGFR2^+$ cells were defined as EPCs. Data were processed using CELL Quest software for the Macintosh (BD). The instrument setup was optimized daily by analyzing the expression of peripheral blood lymphocytes labeled with anti–CD4 APC/CD8 PE/CD3 FITC/CD45 PerCP four-color combination. One trained operator, who was blind to the patients' clinical status, performed all the tests throughout the study.

Statistical Analysis

The means and SDs of all variables were calculated. The circulating EPC count was calculated as the absolute number of cells per μL. The statistical analysis of variance of groups was performed using a one-way ANOVA followed by Fisher's test for the comparison of variance values in the single observation times. A value of $p<0.05$ was considered statistically significant. Correlations between the number of EPCs and serum adhesion molecules were assessed by calculating the weighted means of EPC count and each serum adhesion molecule concentration observed at the same time points. It was thus possible to examine, and closely link, the values of the two dependent variables considered. Statistically significant associations between the numbers of cells weighted and the concentration of serum adhesion molecules were evidenced using linear regression analysis. Correlations between the number of HSCs and EPCs and the concentration of adhesion molecules were assessed using Pearson's coefficient (r). SPSS 11.0 statistical software (SPSS Institute, Cary, USA) and Microsoft Excel were used for tabulation and analysis. Some graphs were constructed using Prism Statistical software (version 4.00; Graphpad, San Diego, USA).

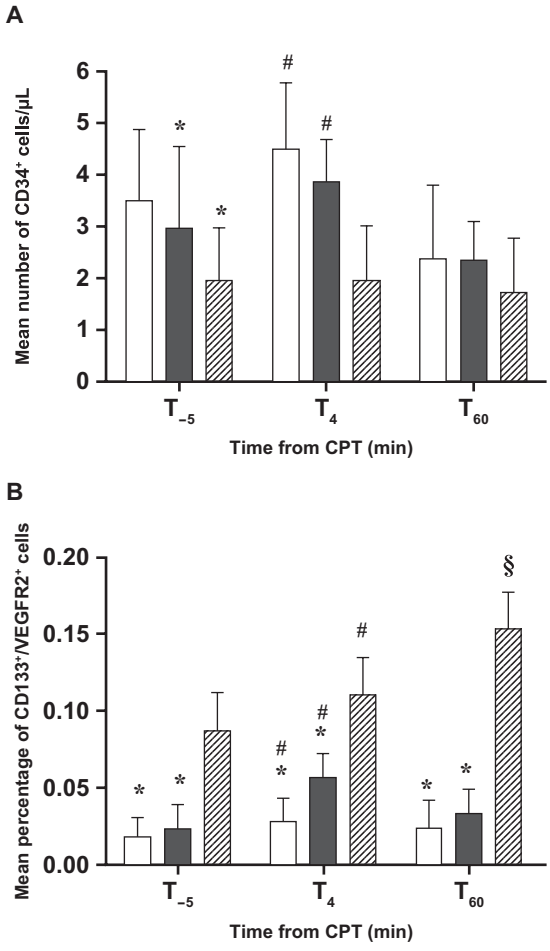

Fig. 2. *Numbers of endothelial progenitor cells at two different maturative stages ($CD34^+$ and $CD133^+/VEGFR2^+$) in healthy subjects (white columns), hypertensive patients (black columns), and patients on chronic kidney disease (hatched columns), 5 min before (T_{-5}), 4 min after (T_4) and 60 min after (T_{60}) cold pressor test. A: Mean of $CD34^+$ cells per microliter±SDs. *$p<0.01$ healthy and hypertensive vs. CKD subjects; $^{\#}p<0.01$ T_4 vs. T_{-5}. B: Mean of the percentage of $CD133^+/VEGFR2^+$ cells±SDs. *$p<0.01$ healthy and hypertensive vs. CKD subjects; $^{\#}p<0.01$ T_4 vs. T_{-5}; $^{\S}p<0.01$ T_{60} vs. T_4.*

Results

Basal Concentration of EPCs

CKD patients had significantly lower basal $CD34^+$ cell levels than healthy controls and hypertensive patients (1.95±1.02 *vs*. 3.49±1.37, $p<0.01$; 1.95±1.02 *vs*. 2.96±1.58, $p<0.01$) (Fig. 2A). On the other hand, at the beginning of the test the percentage of $CD133^+/VEGFR2^+$ cells in CKD subjects was significantly higher than that in controls and hypertensive

Table 2. Endotelial Adhesion Molecules and Blood Pressure Levels 5 min before (T_{-5}) and 4 min after (T_4) and 60 min after (T_{60}) Cold Pressor Test

Time	sICAM-1 (ng/mL)	sVCAM-1 (ng/mL)	sE-selectin (ng/mL)	SBP (mmHg)	DBP (mmHg)	Epinephrine (nmol/L)	Norepinephine (nmol/L)
Basal dosage (T_{-5})							
Healthy subjects	262±96	625±100	61±10	123±14	83±10	0.075±0.006	1.08±0.080
Hypertensive	380±521*	720±180*	75±10*	168±23*	103±31*	0.12±0.014*	1.24±0.14*
CKD	510±721*	2,101±300*	80±30*	135±26*	85±19	0.17±0.06*	1.30±0.17*
After CPT (T_4)							
Healthy subjects	330±902#	760±100#	85±16#	160±10#	100±13#	0.16±0.006#	1.60±0.086#
Hypertensive	480±801*,#	825±200*,#	99±14*,#	189±9*,#	125±23*,#	0.20±0.016*,#	2.20±0.16*,#
CKD	603±921*,#	2,379±200*,#	110±34*,#	167±16*,#	110±10*,#	0.24±0.06*,#	2.24±0.18*,#
After 60 min (T_{60})							
Healthy subjects	280±137	599±100	60±19	119±17	86±13	0.084±0.019	1.10±0.16
Hypertensive	410±911*	691±190*	71±23*	160±19*	99±29*	0.10±0.06*	1.20±0.18*
CKD	581±881*	2,204±700*	139±46*	140±21*	89±23*	0.15±0.09*	1.27±0.18*

SBP, systolic blood pressure; DBP, diastolic blood pressure. Values are mean±SD. *$p<0.05$ *vs*. healthy subjects; #$p<0.05$ *vs*. basal value. sICAM-1, serum intercellular cell adhesion molecule-1; sVCAM-1, serum vascular endothelial cell adhesion molecule-1; CKD, chronic kidney disease; CPT, cold pressor test.

patients (0.08±0.0251% *vs*. 0.018±0.0127%, $p<0.01$; 0.08±0.0251% *vs*. 0.02±0.0158%, $p<0.01$) (Fig. 2B).

Effect of CPT on EPCs

Analysis of the data immediately after CPT (4 min) revealed a slight increase in the number of $CD34^+$ cells in every study group. This increase was statistically significant with respect to basal values only in healthy and hypertensive subjects (4.49±1.28 *vs*. 3.49±1.37, $p<0.01$; 3.86±0.82 *vs*. 2.96±1.58, $p<0.01$). After 60 min, $CD34^+$ levels were reduced to T_0 levels in every group (Fig. 2A). The percentage of CD133 cells that expressed VEGFR2 ($CD133^+/VEGFR2^+$) increased significantly with respect to T_0 in each group just after CPT (0.028±0.015 *vs*. 0.018±0.0127%, $p<0.01$; 0.0567±0.015 *vs*. 0.0233±0.0158%, $p<0.01$; 0.110±0.024% *vs*. 0.08±0.0251%, $p<0.01$). This increase was significantly greater in CKD patients than in hypertensive and healthy subjects ($p<0.01$). In the healthy and hypertensive group, 60 min after CPT, $CD133^+/VEGFR2^+$ returned to basal levels (0.024±0.0180% and 0.0333±0.0157%, respectively). On the other hand, in CKD patients the number of $CD133^+/VEGFR2^+$ maintained an increasing trend, reaching twice the level at T_0, and showed a significant difference with respect to T_4 (0.153±0.023 *vs*. 0.110±0.024%, $p<0.01$) (Fig. 2B).

Blood Pressure Measurement and Arterial Catecholamine Responses

CPT had significant effects on blood pressure, heart rate, and plasma catecholamines in all three groups. After 4 min, the CPT had increased arterial blood pressure and plasma catecholamines in all groups ($p<0.05$). At 60 min, all these values had returned to basal levels (see Table 2 for blood pressure measurement).

Endothelial Adhesion Molecules

The basal concentrations of intercellular cell adhesion molecule-1 (ICAM-1), vascular endothelial cell adhesion molecule-1 (VCAM-1), and E-selectin were significantly higher in patients with essential hypertension and CKD than in healthy subjects. The basal level of adhesion molecules was significantly higher in CKD patients than in hypertensive patients. Adhesion molecule levels were increased at 4 min after CPT in each group. At 60 min, ICAM-1, VCAM-1, and E-selectin values returned to basal levels in healthy and hypertensive groups, whereas E-selectin values increased in CKD patients; meanwhile, VCAM-1 maintained high concentrations in CKD patients (Table 2). Our findings were confirmed and strengthened by the fact that, at every measurement time, the numbers of $CD34^+$ and $CD133^+/VEGFR2^+$ cells were adjusted for the respective concentrations of ICAM-1, VCAM-1, and E-selectin. The pattern in CKD was different from that in the other groups when we weighted the $CD34^+$ or the $CD133^+/VEGFR2^+$ count for ICAM-1,VCAM-1, and E-selectin. These values continued to increase until 60 min after CPT (Fig. 3). Moreover, in our population, a close positive correlation, assessed by Pearson's coefficient (r), was found between the mean for the $CD34^+$ and $CD133^+/VEGFR2^+$ counts on the one hand and, on the other, the concentrations of adhesion molecules in healthy and hypertensive groups. This close correlation with endothelial adhesion molecules was not found in CKD patients.

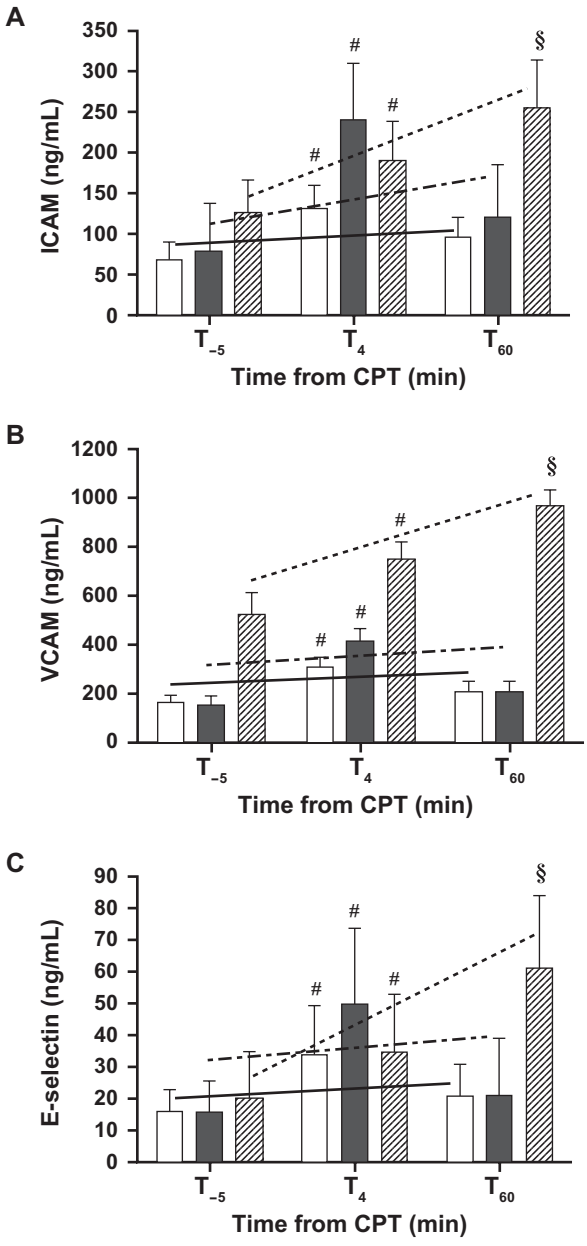

Fig. 3. *Graphic representation of the number of endothelial progenitor cells addressed to endothelial lineage ($CD133^+/VEGFR2^+$) adjusted for single endothelial adhesion molecules (ICAM, VCAM, and E-selectin) and respective regression lines. Healthy subjects (white columns, regression line is a continuous solid line), hypertensive patients (black columns, regression line is a dot-tract line), and patients with chronic kidney disease (hatched column, regression line is dotted line): A: ICAM; B: VCAM; C: E-selectin. $^{\#}p < 0.01$ T_4 vs. T_{-5}; $^{\S}p < 0.01$ T_{60} vs. T_4.*

Discussion

In the present study, HSCs and EPCs were quantified after hemodynamic stress by applying the CPT. The findings confirm the following: 1) immediate vascular stress causes a sudden and marked variation in the number of progenitor cells; 2) a parallel variation that also occurs in adhesion soluble molecules indicates endothelial activation; 3) the pattern in patients with diseases is characterized by endothelial dysfunction and is different from that in healthy subjects.

Characterization of Hematopoietic and EPCs

The repair process following endothelial damage occurs in three phases: 1) mobilization from bone marrow; 2) homing in on the sites of vascular injury; 3) incorporation of the endothelium into the injured blood vessels (*12*).

While maturing in the circulation, HSCs and EPCs are characterized by the gradual expression and then disappearance of surface markers. In the present study, we searched for two cell types: $CD34^+$ and $CD133^+/VEGFR2^+$. CD34, an early marker, is present on HSCs, albeit not exclusively, and is also present in smaller quantities in mature endothelial cells (*13*). In a recent paper, we showed that $CD133^+/VEGFR2^+$ isolated from the maternal circulation increases as the gestational trimesters progress, and that pregnant women with a diagnosis of gestational diabetes and hypertension have higher concentrations of $CD133^+/VEGFR2^+$ than healthy pregnant women (*10*).

Basal Levels of Progenitor Cells

In CKD patients, we suspended erythropoietin treatment in order to obviate any interference in the proliferation and differentiation of progenitor cells following the observations made by Bahlmann *et al. (14)*. Our findings confirm that the CKD patients have significantly fewer CD34 cells than the control population, but the number is comparable to that in hypertensive patients (*15*). One explanation for this may be that apoptosis is increased in progenitor cells. In CKD patients, various cell lines show a tendency toward apoptosis, and the presence of known anti apoptotic stimuli, such as bcl-2, is reduced. This behavior has been attributed to an increase in oxidative stress following an alteration in the balance between the production of oxidating substances and the activity of antioxidating systems (*16*, *17*).

A circulating endothelial microparticles assay recently revealed a dramatic increase in endothelial cell death in patients with CKD (*18*). After quantifying $CD34^+$ cells, we quantified $CD133^+/VEGFR2^+$ cells. Surprisingly, in CKD patients most $CD34^+$ cells differentiated toward endothelial cells, not to the hematopoietic lineage. This underlines the increase in $CD133^+/VEGFR2^+$ cells in CKD patients with respect to control subjects, as well as the reduction with

respect to hypertensive patients. Our findings appear to contradict those reported by other authors, such as Heiss *et al*. and Vasa *et al*.; both of those groups related reduced numbers of EPCs to a higher cardiovascular risk, but this may be explained by the functional impairment present in our patients, and the impaired tendency of these cells to form colonies (*19*, *20*). Hill *et al*. pointed out that a high number of EPCs does not mean a higher ability to repair damage. Increased cardiovascular risk factors were inversely correlated with cell function, thus EPCs have a reduced tendency to form cell colonies (*21*). The "failed" repair of EPCs may cause a higher number of HSCs to convert to EPCs. Our findings are in agreement with those of Imanishi *et al*., who recently demonstrated a hypertension-induced EPC senescence, which might affect the process of vascular remodeling (*22*). Accordingly, Delva *et al*. recently found that plasma total and LDL cholesterol are independent predictors of reduced numbers of circulating EPCs in essential hypertension patients (*23*).

Effect of Cold Pressor Test on EPCs

The findings of the present study confirm the hypothesis that bone marrow responds to injury by releasing a greater number of progenitor cells into the circulation as an immediate response to endothelial mechanical stress. We performed the CPT, which has often been used in the literature for the diagnosis of cardiovascular reactivity in normotensive and hypertensive subjects (*24*). Recently we found elevated numbers of EPCs in patients undergoing hemodialysis, reflecting the direct response of bone marrow to hemodynamic stress on vessels (*10*). Other authors have already demonstrated an increase in stem cells about to undergo endothelial maturation following different pathological vascular stress inductor stimuli (*25*). An increase in acute arterial blood pressure after CPT can expose vessels to mechanical forces. Increased blood pressure directly increases radial stress and the viscous drag that flowing blood exerts on the endothelial lining. Furthermore, the arterial wall is influenced by pressure-related forces such as longitudinal, circumferential, and radial wall stresses (*5*). CPT might induce hemodynamic stress whose severity and efficacy are limited in time. This might explain why the increase in progenitor cells disappeared within 60 min in our hypertensive patients and controls. In CKD patients, on the other hand, an increasing trend was observed, and this continued after 60 min. This finding is in agreement with that of Herbrig *et al*., who found a markedly altered migratory activity of EPCs in patients with ESRD compared to a healthy population, with adhesion to the protein matrix and to endothelial cells. Those authors also observed an increase in the total number of EPCs (*26*). In accordance with our findings, Rodríguez-Ayala *et al*. recently found a discrepancy between HSC and EPC numbers in patients with CKD (*27*). A large body of evidence demonstrates that cardiovascular risk factors induce endothelial injury and endothelial function damage. In parallel, the repair mechanisms for endothelial damage are impaired in these patients, and high levels of EPCs express this complicated capacity to home in on the sites of vascular lesion.

Endothelial Adhesion Molecules and CPT

We measured the concentrations of the three main endothelial-expressed cell adhesion molecules associated with leukocyte activation. The adhesion of monocytic lineage cells on the vascular wall, which depends on their interaction with endothelial cells, is mediated by cell adhesion molecules, including ICAM-1, E-selectin, and VCAM-1. Some authors suggest that increases in the soluble form of adhesion molecules found in association with endothelial dysfunction might be considered a marker of endothelial cell activation (*28*). E-Selectin is a member of the selectin family of cell adhesion molecules, and the ICAM-1 and VCAM-1 belong to an immunoglobulin super family. The surface expression of these cell adhesion molecules is associated with their shedding into the peripheral circulation. Our findings confirm that high basal levels of endothelial adhesion molecules are present in hypertensive and CKD patients (*29*) and that CPT induces a rapid increase in these molecules in all categories (*7*). These observations are in agreement with those recently reported by Nishiwaki *et al*., who found that endothelial E-selectin plays a crucial role in EPC endothelial interaction *in vitro* (*30*). In conclusion, adhesion molecule levels may be considered a marker of endothelial activation in response to hemodynamic trauma altering the vascular surface. EPC and adhesion molecule levels increase rapidly after sudden hemodynamic stress from CPT. There is close contact between bone marrow and vessels mediated by swift cytokine release in the blood circulation. Moreover, CPT has a marked effect on the vessels of patients with diseases causing endothelial dysfunction, such as hypertension and chronic kidney disease. The recruitment of EPCs and the release of adhesion molecules are severely impaired in CKD patients with respect to other groups, thus suggesting new mechanisms underlying the increased cardiovascular risk in this population. This may have an important impact on the treatment of these patients, leading to the discovery of new therapeutic tools.

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