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Original Research Report

Mobilization of Putative High-Proliferative-Potential Endothelial Colony-Forming Cells during Antihypertensive Treatment in Patients with Essential Hypertension

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ABSTRACT

Recent studies have shown that in response to vascular damage or ischemia, bone marrow-derived endothelial progenitor cells (EPCs) are recruited into the circulation. To investigate whether antihypertensive treatment has an influence on the number of circulating EPCs, patients with essential hypertension were treated either with the angiotensin receptor antagonist telmisartan, the calcium channel blocker nisoldipine, or their combination for 6 weeks. At baseline and after 3 and 6 weeks of treatment, EPCs were identified and quantified by fluorescence-activated cell sorting (FACS) analysis and by their capacity to generate colony-forming units of the endothelial lineage (CFU-EC) in a methylcellulose-based assay. During treatment, patients in the nisoldipine groups, but not in the telmisartan group, showed a significant mobilization of EPCs, which in part had the capacity to generate large-sized colonies comprising more than 1,000 cells. Moreover, a remarkable correlation between the number of CFU-EC and the number of circulating CD133⁺/CD34⁺/CD146⁺ cells was observed, thereby providing strong evidence that cells with this phenotype represent functional EPCs. No correlation was found between the numbers of CFU-EC and the blood pressure levels at any time point during the treatment. Hence, nisoldipine-induced mobilization of EPCs might represent a novel mechanism by which this antihypertensive compound independently of its blood pressure-lowering effect contributes to vasoprotection in patients with essential hypertension.

INTRODUCTION

ADULT BONE MARROW contains a variety of hematopoietic and nonhematopoietic stem and progenitor cells, among which a scarce population of endothelial progenitor cells (EPCs) exists. These precursors are characterized by expression of the stem cell markers CD34, CD133, and vascular endothelial growth factor receptor-2 (VEGFR-2) (1,2). Because CD34 and VEGFR-2 are also expressed on mature endothelium, CD133 is currently the only marker

to distinguish EPCs from differentiated endothelial cells, from which it is absent (3–5). Nevertheless, identification of EPCs is still complicated by the fact that all three markers mentioned can also be found on hematopoietic stem cells (5–8). Thus, further phenotypic characterization is needed for the discrimination between EPCs and hematopoietic precursors. Co-expression studies, including surface molecules that are expressed on mature endothelial cells but not on mature hematopoietic cells, provide a useful approach in this context. However, endothelial cells and he-

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matopoietic cells share expression of several antigens, making it difficult to find an endothelial-specific marker. The cell adhesion molecule CD146 (Endo-1 or PIH12), although not exclusively expressed on endothelial cells, might represent a suitable marker to define further the phenotype of EPCs because it is not expressed on hematopoietic cells (9). Besides phenotypical analysis, EPCs can be indirectly identified by their capacity to form colonies in vitro (1,10,11).

Under steady-state conditions, EPCs represent less than 0.01% of the cells in the circulation, but their number can significantly increase in pathological situations, such as vascular or myocardial injury, early stages of heart failure, and malignant diseases (12–15). Furthermore, EPCs can be mobilized from the bone marrow into the circulation by a variety of growth factors, chemokines, hormones, and drugs, including VEGF, granulocyte-macrophage colony-stimulating factor (GM-CSF), estrogen, and statins and subsequently promote neovascularization in ischemic organs or contribute to (re)endothelialization of implanted vascular grafts or denuded arteries (1,16–20). It has therefore been hypothesized that EPCs may play a crucial role in maintaining the integrity of the vascular endothelial monolayer (21).

Hypertension is a highly prevalent risk factor of cardiovascular disease, which is accompanied by alterations of the endothelium and structure of the vascular system (22). Moreover, hypertension is commonly associated with increased levels of angiotensin II, the main effector peptide of the renin angiotensin system, which has been reported to induce EPC proliferation and network formation (23). Although the role of EPCs in vascular biology has been studied extensively, their role and function in hypertension is yet undefined. In this context, the effect of antihypertensive drugs, e.g., angiotensin receptor antagonists or calcium channel blockers, on mobilization of EPCs in patients with essential hypertension has not been examined so far. Therefore, we performed a clinical study to investigate the effect of the angiotensin receptor antagonist telmisartan, the calcium channel blocker nisoldipine, and their combination on the number of circulating EPCs in patients with essential hypertension.

In this study, we provide evidence that treatment of hypertensive patients with nisoldipine leads to mobilization of EPCs, which in part exhibit exceptional clonogenic capacity and are characterized by a CD133⁺/CD34⁺/CD146⁺ phenotype.

MATERIALS AND METHODS

Patients and study protocol

The study comprised 37 patients with essential hypertension and was performed as a single-center, random-

ized, blinded study. The study was approved by the local Ethics Committee and conducted according to the principles of Good Clinical Practice and the Declaration of Helsinki. Patients were eligible for the study if they had been diagnosed with essential hypertension (i.e., exclusion of other etiologies of hypertensive disease), were between 20 and 80 years old, and had signed the informed consent form. Important exclusion criteria were treatment with statins or hormones (estrogens, gestagens), liver or renal dysfunction, severe heart failure, diabetes mellitus, acute or unstable coronary artery disease, or previously experienced hyperreactivity against angiotensin receptor antagonists or calcium channel blockers. Patients who were on continuous antihypertensive treatment underwent a wash-out phase of all antihypertensive and vasoactive medication during 2 weeks prior to study entry. During this period, clonidine and hydrochlorothiazide were available as rescue medication in the event that blood pressure reached a level of 180/110 mmHg.

Baseline examination at day 1 of the study included peripheral blood analysis, physical examination, and a 24-h automated ambulatory blood pressure monitoring. Study medication was started at day 2 and was either telmisartan 40 mg orally per day, nisoldipine 10 mg orally per day, or a combination of telmisartan 40 mg plus nisoldipine 10 mg orally per day for 3 weeks. Evaluation at day 21 comprised peripheral blood analysis and 24-h ambulatory blood pressure monitoring. At this time, the daily dose of the study medication was increased to 80 mg in the telmisartan group, to 20 mg in the nisoldipine group, and to 80/10 mg for the combined therapy with telmisartan/nisoldipine; this dosage was given for the following 3 weeks. The final examination at day 42 again consisted of peripheral blood analysis and 24-h ambulatory blood pressure monitoring. Thereafter, the initial antihypertensive regimen was restarted and patients were supervised until their blood pressure had reached levels similar to those before study entry.

Preparation of peripheral blood samples

Heparinized blood samples obtained from patients at baseline, day 21, and day 42 were incubated with hemolytic buffer (0.155 mol/L NH₄Cl, 0.012 mol/L NaHCO₃, 0.1 mmol/L EDTA, pH 7.2) for 5 and 2 min and immediately prepared for fluorescence-activated cell sorting (FACS) analysis and colony assays.

Flow cytometry analysis

After washing in phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA), 1 × 10⁶ cells per tube were incubated with phycoerythrin (PE)-conjugated anti-CD133 monoclonal antibody (mAb; clone AC141, Miltenyi Biotec, Bergisch Gladbach,

Germany) in combination with either fluorescein isothiocyanate (FITC)-conjugated anti-CD34 mAb (Anti-HPCA-2, BD Pharmingen, Hamburg, Germany), anti-CD45 mAb, anti-CD14 mAb (all from BD Pharmingen), or anti-von Willebrand Factor (vWF; Serotec, Düsseldorf, Germany). Other combinations included PE-anti-CD144 mAb (Coulter-Immunotech, Krefeld, Germany) with either FITC-anti-CD34 or FITC-anti-vWF, or PE-anti-CD146 (Coulter-Immunotech) with either FITC-anti-CD34 or FITC-anti-vWF. Isotype-matched anti-immunoglobulins mAbs (BD Pharmingen) served as controls. All incubations were performed at 4°C in the presence of normal goat serum. For expression studies including vWF, cells were fixed and permeabilized (Intrastain Kit, Dako Cytomation, Hamburg) before incubation with anti-vWF mAb. Two-color flow cytometry was accomplished using a FACS Calibur flow cytometer (Becton Dickinson, Heidelberg, Germany) and Cell Quest software (Becton Dickinson) as previously described (2). Each analysis included at least 50,000 events.

Clonogenic assay for EPCs

Remaining peripheral blood cells were plated at 1×10^5 cells/ml in methylcellulose (Stem Cell Technologies, St. Katharinen, Germany), as previously described (2). Briefly, cells were cultured in methylcellulose that was supplemented with stem cell growth factor (SCGF; 100 ng/ml, TEBU, Frankfurt, Germany) and VEGF (50 ng/ml, TEBU). All cultures were performed in quadruplicate, incubated at 37°C in 5% CO₂ and 95% humidity, and scored after 14 days using an inverted microscope.

Statistical analysis

Distribution of data was tested with the Kolmogorov–Smirnov test. Continuous variables were expressed as arithmetic mean \pm standard deviation (SD) if normally distributed or otherwise as median with 25% and 75% percentiles (IQR). Differences in baseline characteristics among groups were tested with one-way analysis of variance (ANOVA) if normally distributed, while baseline differences of variables with skewed distribution were tested with the Kruskal–Wallis *H* or Mann–Whitney *U* test. Changes during treatment within different treatment groups were tested either with repeated-measure ANOVA followed by the paired *t*-test, the nonparametric test of repeated measures on ranks (Friedman test) followed by Wilcoxon’s test, or with Wilcoxon’s test alone. Bivariate correlations were analyzed using Spearman’s rho. Probability values <0.05 were considered significant. For all statistical analysis, SPSS version 12.0 was used.

RESULTS

Patients’ characteristics

Baseline characteristics of the hypertensive patients studied are shown in Table 1. Treatment groups did not significantly differ in age, gender, mean blood pressure, or washout medication, but patients randomized to receive nisoldipine had a significantly higher body mass index (BMI) as compared to patients who had been randomized to telmisartan treatment (27.1 ± 4.1 vs. 23.0 ± 3.0 ; $p < 0.05$). Except for this observation, baseline characteristics

TABLE 1. PATIENT CHARACTERISTICS

	Telmisartan (n = 12)	Nisoldipine (n = 13)	Combination (n = 12)	P
Gender (male/female)	7/5	6/7	7/5	ns.
Age (years)	59.0 \pm 7	56.9 \pm 8	59.6 \pm 8	ns.
Mean blood pressure (mmHg)	103.6 \pm 8.7	108.6 \pm 12.5	99.8 \pm 7.5	ns.
Body-mass index (kg/m ²)	23.0 \pm 3.0*	27.1 \pm 4.1*	24.5 \pm 4.2	*0.043
Cholesterol (mg/dL)	244 \pm 32	236 \pm 48	254 \pm 46	ns.
HDL (mg/dL)	63 \pm 13	56 \pm 11	58 \pm 16	ns.
LDL (mg/dL)	158 \pm 25	150 \pm 41	164 \pm 28	ns.
C-reactive protein high sensitive (mg/L)	0.95 (0.7-1.925)	2.1 (1.25-3.7)	1.9 (1.125-3.15)	ns.
Creatinine (mg/dL)	0.95 \pm 0.17	0.95 \pm 0.15	0.93 \pm 0.18	ns.
Fasting glucose (mmol/dL)	5.1 \pm 0.6	5.4 \pm 0.4	5.4 \pm 0.7	ns.
Current Smokers	0	1	1	ns.
Wash-out medication:				
Hydrochlorothiazide	6	8	7	ns.
Clonidine	2	2	1	ns.

Data are given as mean \pm SD (parametric data) or median with interquartile range (non-parametric data). n.s.; not significant.

TABLE 2. EFFECT OF ANTIHYPERTENSIVE TREATMENT ON BLOOD PRESSURE AND BIOMARKERS

	<i>Telmisartan</i>			<i>Nisoldipine</i>			<i>Combination</i>			<i>P</i>
	<i>Baseline</i>	<i>Day 21</i>	<i>Day 42</i>	<i>Baseline</i>	<i>Day 21</i>	<i>Day 42</i>	<i>Baseline</i>	<i>Day 21</i>	<i>Day 42</i>	
Mean blood pressure (mmHg)	103.6 ± 8.7	99.9 ± 9.4	96.6 ± 12.5 [#]	108.6 ± 12.5	103.5 ± 10.7	104.7 ± 9.7	99.8 ± 7.5	94.9 ± 6.6 [#]	91.6 ± 5.3 [#]	[#] <0.05 vs. baseline
Biomarker cGMP (pmol/mL)	10.6 ± 7.3	13.0 ± 9.5	14.4 ± 7.5	6.1 ± 3.2	13.1 ± 10.9*	17.5 ± 25.9*	9.5 ± 5.7	15.5 ± 13.9	12.9 ± 6.1	*<0.05 vs. baseline
MMP-9 (ng/mL)	1.27 ± 0.73	1.38 ± 0.62	1.22 ± 0.66	1.55 ± 1.17	1.53 ± 1.06	1.68 ± 1.11	1.29 ± 0.67	1.32 ± 0.50	1.20 ± 0.42	ns.
MCP-1 (pg/mL)	171 ± 50	172 ± 43	171 ± 75	166 ± 45	166 ± 48	163 ± 56	168 ± 53	148 ± 61	149 ± 32	ns.

Data are given as mean ± SD. cGMP was creatinine-indexed to correct for differences in renal urinary volumes. ns.; not significant.

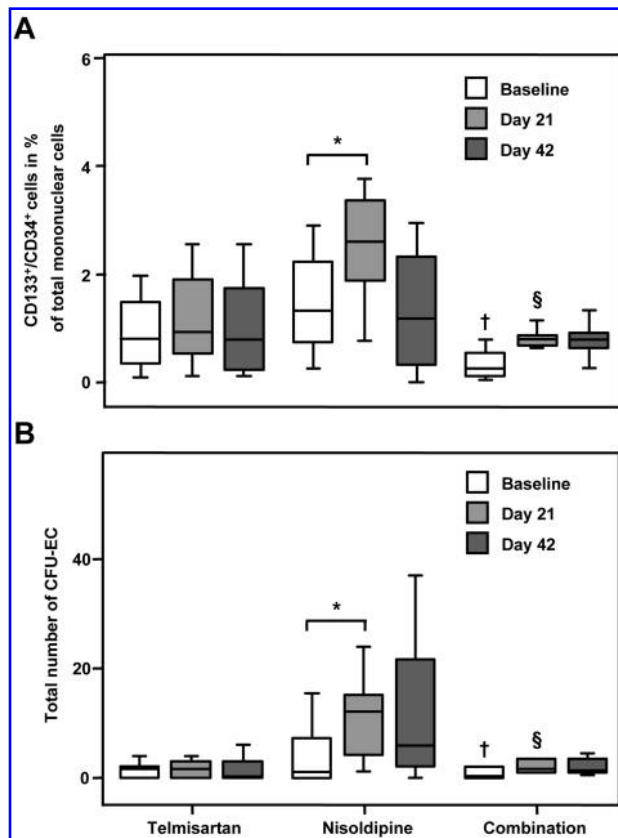


FIG. 1. Mobilization of EPCs during antihypertensive treatment as identified by FACS analysis and assessment of endothelial colony formation. **(A)** The quantity of CD133⁺/CD34⁺ cells as revealed by flow cytometry is expressed as percentage of total mononuclear blood cells. Values at baseline, after 21 days, and 42 days of antihypertensive treatment are given for the different treatment groups. **(B)** Total numbers of CFU-EC generated from mononuclear blood cells in vitro at baseline, at day 21, and at day 42 of antihypertensive treatment are shown for the different groups. Median, lower quartiles (Tukey's hinges), and extremes are given. * $p < 0.01$ versus baseline levels in the nisoldipine group; † $p < 0.05$ versus baseline levels in the nisoldipine group; § $p < 0.05$ versus baseline levels in the combination group.

showed no clinically relevant differences among treatment groups. As shown in Table 2, mean blood pressure was reduced in patients after 6 weeks of antihypertensive treatment with telmisartan (-7.0 mmHg; $p < 0.05$) and the combination of both substances (-8.2 mmHg; $p < 0.05$). In patients treated with nisoldipine alone, a trend toward lower blood pressure values was observed, but values failed to reach statistical significance during the study period (-3.9 mmHg; $p = 0.090$).

Mobilization of EPCs during antihypertensive treatment

To detect circulating EPCs, peripheral blood cells were analyzed by flow cytometry for the presence of

CD133⁺ and CD34⁺ cells. Additionally, blood cells were transferred to a methylcellulose-based assay, which has been shown to specifically induce formation of endothelial colonies (referred to as CFU-EC) from granulocyte colony-stimulating factor (G-CSF) mobilized CD133⁺/CD34⁺ EPCs (2). In this assay, peripheral blood cells from healthy controls only rarely give rise to single colonies, according to the finding that the mononuclear cell fraction from peripheral blood of healthy donors contains less than 0.01% CD133⁺ endothelial progenitor cells (12–15). As observed for the number of circulating CD133⁺/CD34⁺ cells (Fig. 1A), the absolute number of colony-forming units–endothelial (CFU-EC) at baseline in patients randomized to nisoldipine treatment was significantly higher than those obtained in the combination group [median 1 (range 0–8) vs. 0 (0–2), $p < 0.05$; Fig. 1B], but was not significantly different from the levels noted in the telmisartan group [2 (0–2)]. During treatment, a significant increase in the number of CFU-EC was found at day 21 in patients who received nisoldipine (mono and combination therapy) but not in patients treated with telmisartan alone [nisoldipine: 12 (1–24), $p < 0.01$ vs. baseline; combination: 2 (1–4), $p < 0.05$ vs. baseline]. Furthermore, a nonsignificant trend toward increased CFU-EC numbers at day 42 in nisoldipine-treated patients but not in patients treated with telmisartan alone was observed [nisoldipine: 5 (2–37), $p = 0.094$; combination: 1.5 (1–4); $p = 0.058$]. In a similar manner, the number of CD133⁺/CD34⁺ cells showed a significant increase at day 21 in patients receiving nisoldipine and returned to nonsignificant levels at day 42 of treatment (Fig. 1A). Importantly, numbers of circulating EPCs did not show any correlation with clinical parameters, such as BMI (data not shown) and blood pressure values, at any time point (Fig. 2).

Phenotypic characterization of mobilized EPCs

Statistical analysis revealed a significant correlation between the level of circulating CD133⁺/CD34⁺ cells and the number of generated CFU-EC ($\rho = 0.41$; $p < 0.05$). To further identify the surface molecule expression pattern specific for functional EPCs, co-expression of the endothelial markers vWF, VE-cadherin (CD144), CD31, and CD146 on the CD133⁺/CD34⁺ population was studied. As shown in Fig. 3, a strong correlation between the number of CD133⁺/CD34⁺/CD146⁺ cells and the number of generated CFU-EC ($\rho = 0.93$; $p < 0.0001$) was discovered, indicating that this subset represents functional EPCs. No correlation was found between the number of CD133⁺/CD34⁺ cells co-expressing either vWF, VE-cadherin, or CD31 and the quantity of endothelial colonies (data not shown).

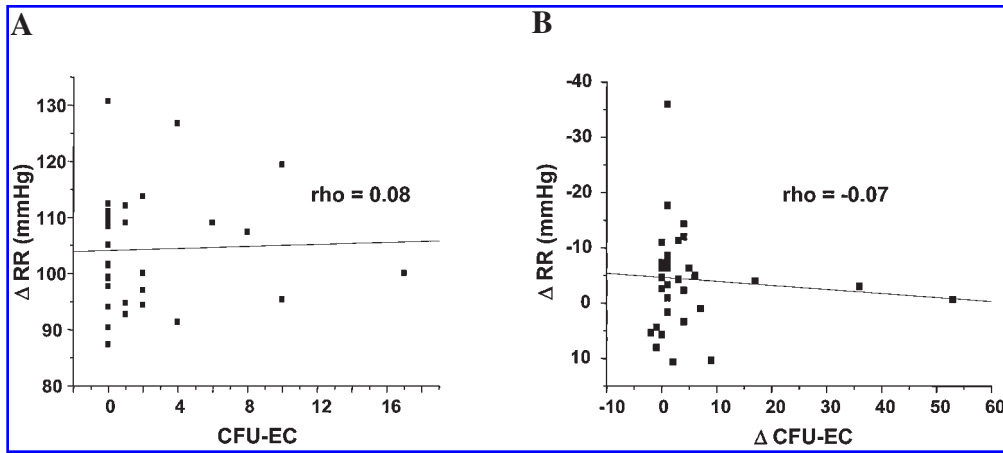
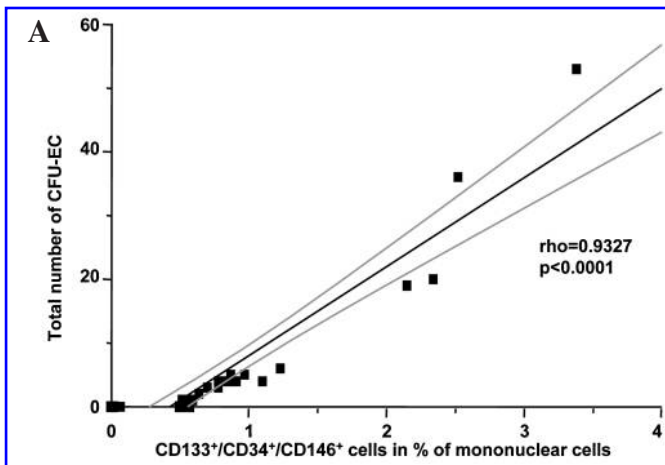


FIG. 2. The number of endothelial colonies (CFU-EC) does not correlate significantly with blood pressure or blood pressure reduction during antihypertensive treatment. **(A)** Correlation between the number of CFU-EC and mean arterial blood pressure at baseline. **(B)** Correlation between the change in the number of CFU-EC and change in mean arterial blood pressure at day 21 of antihypertensive treatment. Correlations are given as Spearman's rho, as indicated.



Morphological characteristics of in vitro-generated CFU-EC

Colonies grown in methylcellulose were not only evaluated for their number but also for their morphology. With respect to the size, three different classes of CFU-EC were discernible (Fig. 4). Most of the colonies consisted of 20–100 loosely packed small-sized round cells. In addition, intermediate-sized CFU-EC composed of up to 500 cells and large-sized colonies comprising more than 1000 cells were detected. These giant colonies were exclusively observed in cultures derived from patients treated with nisoldipine alone ($n = 5$ patients).

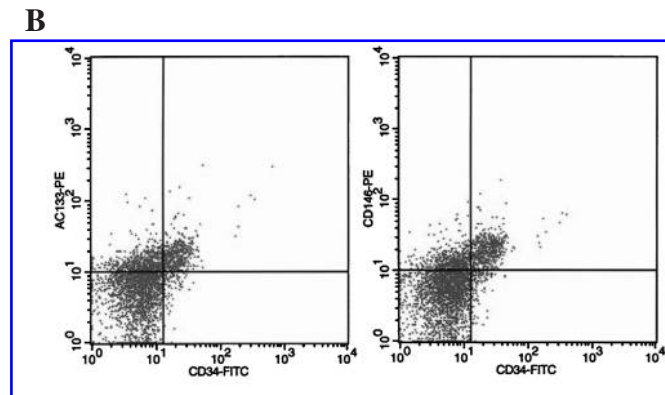


FIG. 3. Mobilized EPCs are characterized by a CD133⁺/CD34⁺/CD146⁺ phenotype. **(A)** Correlation between CFU-EC and CD133⁺/CD34⁺/CD146⁺ cells. Gray lines indicate 95% confidence interval limits for the linear fit (black line). **(B)** Representative two-color flow cytometry analysis of the peripheral blood mononuclear cell fraction from a patient treated with nisoldipine, quantifying co-expression of CD133, CD34, and CD146.

DISCUSSION

The main findings of the present study are that: (1) treatment with the calcium channel blocker nisoldipine leads to a mobilization of EPCs in patients with essential hypertension, (2) mobilized EPCs display a CD133⁺/CD34⁺/CD146⁺ phenotype, and (3) in part possess the capacity to generate extraordinary large-sized colonies.

In our study, EPCs were identified and quantified by two methods. In one approach, EPCs were studied in a clonogenic assay, which has been developed and established in our laboratory (2). This assay has been shown to reflect specifically the number of EPCs contained in CD133⁺ populations and the endothelial nature of the resulting colony-forming units in this assay, so called CFU-EC, has been proven by immunocytochemistry (2). In parallel, FACS analysis was performed using fluorochrome-conjugated antibodies against CD133 and CD34, because EPCs have been shown to be contained in the CD133⁺/CD34⁺ population of bone marrow and

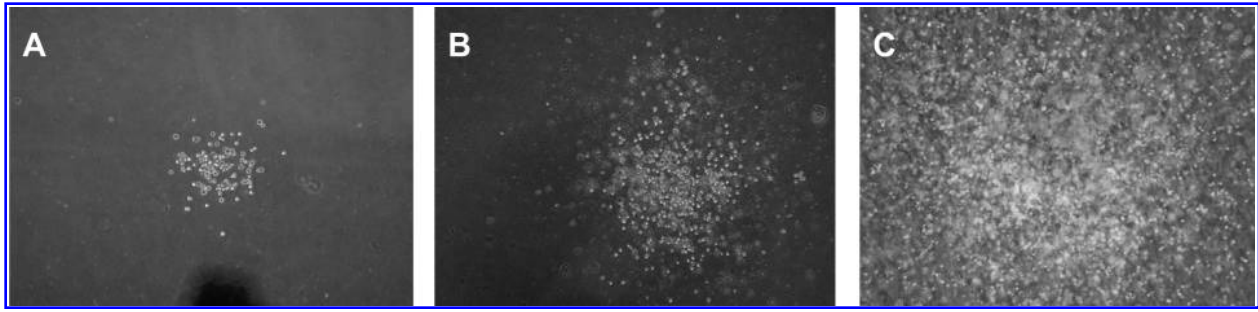


FIG. 4. Morphological characteristics of CFU-EC observed during antihypertensive treatment. Three different sizes of CFU-EC were distinguishable, indicating different proliferative capacities of the mobilized EPCs. **(A)** Small-sized CFU-EC with typical morphology showing 20–100 loosely packed small round cells. **(B)** Intermediate-sized CFU-EC with typical morphology. **(C)** Large-sized CFU-EC with typical morphology. Large-sized CFU-EC were exclusively generated from samples of patients treated with nisoldipine alone. (Original magnification, 10 \times .)

mobilized peripheral blood (1,2). In this context, levels of baseline and mobilized CD133⁺/CD34⁺ cells are consistent with those observed in other clinical studies investigating EPC mobilization (12,24). Circulating CD133⁺/CD34⁺ populations were then further analyzed for coexpression of various endothelial markers, including vWF, VE-cadherin, CD31, and CD146. Expression of VEGFR-2 was not studied because reliable fluorochrome-conjugated antibodies to this molecule were not commercially available at the time when our study was performed and indirect staining combined with direct staining for two-color flow cytometry was found to yield nonspecific results (unpublished observation). Interestingly, by using these two methods, we found a remarkable correlation between the number of CFU-EC generated *in vitro* and the number of CD133⁺/CD34⁺/CD146⁺ cells in the circulation, suggesting that this phenotype defines a specific subset of functional EPCs. This interpretation is in line with a recent study by Delorme and colleagues, demonstrating that the CD146⁺ population of human peripheral blood not only comprises mature endothelial cells but also contains a subset of functional EPCs (25). Our interpretation gains further support by the observation that the other subsets of circulating CD133⁺/CD34⁺ cells detected in our study did not correlate with the number of CFU-EC. Thus, our data might add to the ongoing discussion whether CD146 might be a useful marker to determine biological activity of EPCs (26).

Baseline numbers of *in vitro*-grown CFU-EC as well as those of circulating CD133⁺/CD34⁺ cells showed a considerable interpatient variation, a phenomenon that is common in stem cell research (27). This fact together with the relatively small number of patients enrolled in the study contributed to the considerable level of variability in our data. Despite these limitations, we were able to detect mobilization of EPCs in all patients receiving nisoldipine (alone and in combination with telmisartan),

whereas treatment with telmisartan alone was found not to influence the numbers of circulating EPCs. Furthermore, nisoldipine-induced mobilization of EPCs was shown to peak at day 21 of treatment and a trend toward elevated numbers of EPCs was observed even after 42 days of treatment. Additionally, nisoldipine treatment induced a shift in the antigen expression of the mobilized cells from a progenitor phenotype to a phenotype of mature endothelial cells (data not shown). This shift was detectable at day 42 of the study and was associated with the appearance of morphologically mature endothelial cells in the colony assays (data not shown). Thus, nisoldipine may also favor accelerated differentiation of mobilized EPCs toward mature endothelial cells, which then might contribute to vessel repair and vasoprotection, respectively, in hypertensive patients. Interestingly, induction of EPC mobilization followed by accelerated EPC differentiation has also been demonstrated for growth factors, such as VEGF, and statins (12,19).

In our study, telmisartan significantly lowered blood pressure, whereas nisoldipine only induced a statistically nonsignificant trend toward lower blood pressure. The explanation for this observation remains unclear. Although we cannot exclude that different blood pressure-lowering effects of telmisartan and nisoldipine account for different effects on EPC mobilization, neither baseline levels of circulating EPCs nor numbers of mobilized EPCs during treatment significantly correlated with mean blood pressure levels or the extent of blood pressure reduction at any time point. Therefore, it is rather unlikely that blood pressure reduction is considerably involved in nisoldipine-mediated mobilization of EPCs.

The finding that three different sizes of CFU-EC were distinguishable in our clonogenic assay suggests that mobilized cells comprised EPCs with different clonogenic and proliferative properties. In the hematopoietic system, it is well established that on the basis of these properties, a hierarchy of stem and progenitor cells exists (28). In

this context, colony-forming cells with high proliferative potential (HPP-CFC) have been defined by their ability to form large colonies in vitro (approximately 5×10^4 cells/colony) (29). Recently, Ingram and colleagues reported the identification of a similar hierarchy of EPCs (11). They showed that human cord blood but not adult peripheral blood contains a population of high proliferative potential-endothelial colony-forming cells (HPP-ECFC), which gives rise to higher numbers and larger sizes of colonies and at an earlier time point as compared to adult peripheral blood EPCs. Upon replating, HPP-ECFC had the capacity to generate secondary and tertiary colonies. Additionally, HPP-ECFC-derived cells showed a five-fold higher expansion potential than adult peripheral blood EPCs. Although we did not study the proliferative capacity of the three different classes of colonies and used different culture conditions for our colony assay, we hypothesize that the large-sized colonies observed in our study are derived from HPP-ECFC, which are mobilized from the bone marrow into the circulation. Our findings seem to complement the study by Ingram et al., in which HPP-ECFC could not be identified in steady-state adult peripheral blood. Thus, certain conditions that induce mobilization of EPCs in adult humans might also lead to a release of HPP-ECFC into the circulation. Besides, it is important to note that the colony assay used in our study was developed in close analogy to the standard methylcellulose-based colony assay for hematopoietic stem and progenitor cells and, therefore, might be ideal to investigate the full clonogenic potential of different populations of human EPCs.

Interestingly, large-sized CFU-EC consisting of more than 1,000 cells were only observed in the nisoldipine monotherapy group (5/13 patients), indicating that nisoldipine induces mobilization of HPP-ECFC, whereas telmisartan (alone or combined with nisoldipine) might interfere with this process. It is rather unlikely that nisoldipine itself increased the proliferative properties of mobilized EPCs because also small- and intermediate-sized colonies were observed during treatment with nisoldipine. Mechanisms responsible for nisoldipine-induced mobilization of EPCs remain speculative. A previous report suggests that nisoldipine affects endothelial nitric oxide (NO) bioavailability, a molecule that is known to play a pivotal role in vascular integrity and, recently, has been shown to be essential for EPC mobilization from the bone marrow niche (30,31). Hence, nisoldipine-mediated induction of NO bioavailability in the bone marrow niche might account for increasing EPC numbers during anti-hypertensive treatment with this compound. Second, nisoldipine as well as other dihydropyridines have been shown to induce microvascular permeability and subsequently to cause pretibial edema, a well-known side effect of dihydropyridines (32). Induction of microvascular permeability may also contribute to changes in the

bone marrow niche, e.g., extravasation of matrix-cleaving enzymes, which may ultimately facilitate EPC mobilization. Nevertheless, in our study, numbers of circulating EPCs were not significantly higher in patients developing pretibial edema during treatment with nisoldipine (4 patients; data not shown).

In contrast to nisoldipine treatment, treatment with telmisartan did not significantly affect the total number of circulating EPCs in the patients studied. Furthermore, blood samples from telmisartan-treated patients (mono and combination therapy) did not yield any giant CFU-EC, as observed in patients treated with nisoldipine alone, indicating that telmisartan attenuates nisoldipine-induced mobilization of different population of EPCs. These findings are in line with a recent study by Nickenig and colleagues, which showed that the number of circulating EPCs inversely correlates with the intake of AT1 receptor antagonists in patients with coronary heart disease (33). In this regard, a negative effect of AT1 receptor antagonists on the expression and activity of MMP-9 (gelatinase B) has been reported, a protease that has been implicated in EPC mobilization from the bone marrow niche (34,35). Hence, such potential telmisartan-induced effects may be responsible for the absence of HPP-ECFC in patients who received the combination therapy. Conversely, Bahlmann and colleagues recently reported that AT1 receptor antagonists induce mobilization of circulating endothelial progenitor cells in patients with type 2 diabetes (36). The apparent discrepancy with our findings is most likely due to differences in the type of cells investigated as well as with differences in the disease studied. For identification of EPCs, Bahlmann et al. placed the mononuclear cell fraction from patients with type 2 diabetes in suspension culture containing low concentrations of angiogenic factors and analyzed the adherent cell fraction obtained after 7 days for the number of Ac-DiI-LDL-uptake- and UEA-1-binding-positive cells. Because Ac-DiI-LDL uptake and UEA-1 binding represent functionalities commonly shared by mature endothelial cells, it is impossible to state whether the cells quantified in this study indeed represented EPCs. Nevertheless, our data indicate that treatment with telmisartan does not lead to an increase in the number of circulating EPCs in patients with essential hypertension.

In summary, we demonstrated that nisoldipine induces mobilization of EPCs in hypertensive patients. This effect appeared to occur independently of the blood pressure-lowering effect of this compound. Mobilized EPCs were identified to display a CD133⁺/CD34⁺/CD146⁺ phenotype. With respect to functional properties, a proportion of the mobilized EPCs (exclusively mobilized in the nisoldipine monotherapy group) had the capacity to generate extraordinarily large-sized CFU-EC that to our knowledge have not been described in adult humans be-

fore. In conclusion, nisoldipine-induced EPC mobilization in patients with essential hypertension might represent a novel mechanism by which this compound contributes to vasoprotection in hypertensive patients.

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