Nifedipine Improves Endothelial Function
Role of Endothelial Progenitor Cells

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Abstract—Nifedipine has been shown to improve endothelial function. Recent studies have indicated that endothelial function is correlated with the number of circulating endothelial progenitor cells (EPCs), but it is unclear whether nifedipine affects the number and function of EPCs. The aims of this study were to determine the effects of nifedipine on the number and function of EPCs and to investigate the relationship between improvement of endothelial function and EPC numbers in patients with hypertension. Stage 1 hypertensive men (n = 37) were randomly divided into the nifedipine group and the control untreated group. The nifedipine group was administered slow-release nifedipine (20 mg) once daily. At baseline and after 4 weeks, flow-mediated dilation, blood pressure, biochemical data, and the number of circulating CD34+CD133+ progenitor cells and EPCs were measured. The direct effects of nifedipine on EPC number and function were assessed in vitro. In the nifedipine group, flow-mediated dilation and the numbers of circulating CD34+CD133+ progenitor cells and EPCs were increased, along with a decrease of serum malondialdehyde low-density lipoprotein. The improvement of flow-mediated dilation by nifedipine was correlated with the increase of circulating CD34+CD133+ progenitor cells. Nifedipine also improved angiogenesis-related functions of EPCs (differentiation, migration, and resistance to oxidative stress) in vitro. Thus, nifedipine improved endothelial function and EPC function in stage 1 hypertensive subjects. The latter action may be mediated by reduction of oxidative stress and suppression of EPC apoptosis. These results demonstrate that nifedipine preserves endothelial integrity in patients with hypertension, at least partly, by enhancing EPC numbers and activity. (Hypertension. 2008;52:491-498.)

Key Words: hypertension ■ endothelial function ■ endothelial progenitor cell ■ nifedipine ■ oxidative stress

Nifedipine is a calcium channel blocker that induces vasorelaxation by blocking calcium ion influx into vascular smooth muscle cells. It thereby reduces the systemic blood pressure (BP) and prevents coronary vasospasm.1,2 Furthermore, nifedipine has been shown to preserve endothelial function and to prevent the progression of atherosclerosis in patients with hypertension and/or coronary artery disease.3-6 Experimental studies have revealed that some of these actions are independent of its BP-lowering effects and are related to the promotion of NO release from the endothelium and suppression of reactive oxygen species (ROS) in and around vascular walls.7-10

We reported previously that human endothelial progenitor cells (EPCs) are mobilized into the peripheral circulation from the bone marrow and that circulating EPCs play an important role not only in angiogenesis but also in mending the endothelium of conduit arteries by supplying “fresh” endothelial cells.11-13 Indeed, the number of EPCs has shown an inverse correlation with cardiovascular risk factors and a positive correlation with endothelial function.14-16 Therefore, when evaluating certain drugs for their potential to evoke vascular protection, it seems important to assess the influence of such drugs on both endothelial function and number and functions of circulating EPCs.17 Accordingly, we hypothesized that nifedipine would improve endothelial function in a patient population of stage 1 hypertension by ameliorating the number and functions of circulating EPCs.

Methods
Methods regarding quantification of CD34+CD133+ progenitor cells and EPCs, biochemical analysis, and in vitro analysis, including detection of oxidative stress, are described in detail in the online supplemental data (please see http://hyper.ahajournals.org).

Clinical Study
Thirty-seven consecutive male outpatients with newly diagnosed stage 1 hypertension whose BP had not been normalized after lifestyle modification for ≥3 months were enrolled after informed consent was obtained. Patients with a history of malignancy, cardiovascular events, or active inflammatory disease and those with other cardiovascular risk factors were excluded. The patient population of stage 1 hypertension by ameliorating the number and functions of circulating EPCs.
CPCs were compared between the 2 groups (Figure 1). In the nifedipine group, systolic BP, diastolic BP, and the serum MDA-LDL level were significantly decreased compared with the baseline values (Table). After nifedipine treatment, both CPCs and EPCs increased significantly, and endothelial function was improved (Figure 2). Interestingly, the percent-

Assessment of Endothelial Function

Endothelial function was assessed by measuring endothelium-dependent flow-mediated dilation (FMD) of the brachial artery. FMD was measured noninvasively using a high-resolution ultrasound apparatus with a 7.5-MHz linear array transducer (Prosound SSD-6500SV, Aloka Co Ltd) according to the guidelines of the International Brachial Artery Reactivity Task Force. Endothelium-independent dilation of the brachial artery was quantified at 5 minutes after sublingual administration of glycerol trinitrate (0.15 mg; Nihon Kayaku). Throughout the study, FMD was examined by a single cardiologist who was blinded to the treatment regimen of each subject by using same ultrasound apparatus and probe set. Both FMD and glycerol trinitrate-induced dilation (GTD) were expressed as the percentage of change from the baseline value. To estimate the relative proportion of endothelium-dependent dilation to the maximally achievable dilation because of vascular smooth muscle relaxation, the FMD:GTD ratio was also calculated.

Statistical Analysis

Results are expressed as the means±SEMs. Comparison of continuous variables in the clinical study was performed by Student t test or the paired t test, as appropriate. Correlations between the parameters were assessed by calculating Pearson’s correlation coefficient (r). Comparisons between the in vitro experimental groups were performed using ANOVA followed by Fisher’s protected least significant difference test. In all of the analyses, P<0.05 (2-sided) was considered statistically significant.

Results

Influence of Nifedipine on Endothelial Function (FMD) and CPCs

Baseline characteristics of patients are shown in the Table. After the 4-week study period, the percentage changes of systolic BP, serum malondialdehyde low-density lipoprotein (MDA-LDL), FMD, FMD:GTD ratio, and the number of CPCs were compared between the 2 groups (Figure 1). In the nifedipine group, systolic BP, diastolic BP, and the serum MDA-LDL level were significantly decreased compared with the baseline values (Table). After nifedipine treatment, both CPCs and EPCs increased significantly, and endothelial function was improved (Figure 2). Interestingly, the percent-

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Percentage changes of parameters after the 4-week treatment period in the nifedipine and control untreated groups. Changes of MDA-LDL, FMD, FMD:GTD ratio, and CPCs were significantly greater in the nifedipine group compared to the control untreated group.
Thus, we assessed the effects of nifedipine on oxidative stress in EPCs. Intracellular accumulation of ROS was monitored using a fluorescent dye indicator, dichlorofluorescein diacetate. EPCs showed a significant increase of ROS after exposure to 500 μmol/L of H2O2, and this was inhibited by cotreatment with nifedipine (Figure 5A and 5B).

**Influence of Nifedipine on EPC Differentiation**

Nifedipine stimulated the appearance of EPC-like adherent cells during culture in a concentration-dependent manner (Figure 3). There was ubiquitous expression of both CD31 and vascular endothelial growth factor (VEGF) receptor 2 on EPC-like adherent cells (Figure 4).

**Influence of Nifedipine on ROS Accumulation and Viability of EPCs**

Several studies reported that nifedipine has an antioxidant property.8.10,21,22 Thus, we assessed the effects of nifedipine on oxidative stress in EPCs. Intracellular accumulation of ROS was monitored using a fluorescent dye indicator, dichlorofluorescein diacetate. EPCs showed a significant increase of ROS after exposure to 500 μmol/L of H2O2, and this was inhibited by cotreatment with nifedipine (Figure 5A and 5B).
Effect of Nifedipine on EPC Migration

Migration of EPCs in response to recombinant human VEGF was assessed using a modified Boyden chamber apparatus in the presence or absence of H$_2$O$_2$ (500 μmol/L). H$_2$O$_2$ significantly inhibited the migratory activity of EPCs in response to VEGF. Nifedipine, per se, did not possess a direct chemotactic effect on isolated EPCs without VEGF (data not shown), but nifedipine did aug-

Figure 4. Effects of nifedipine on expression of endothelial lineage markers on EPCs. A, Immunocytochemistry for VEGF receptor 2 (VEGFR2) and CD31 after culture of EPCs in the presence of nifedipine (0.1 or 1.0 μmol/L). Images are shown at ×200 magnification. B, RT-PCR analysis of VEGFR2, CD31, and endothelial NO synthase (eNOS) mRNA expression by EPCs cultured with or without nifedipine (0.1 μmol/L). Nifedipine increased the expression of VEGFR2 mRNA.

Figure 5. Effect of nifedipine on oxidative stress in EPCs exposed to H$_2$O$_2$. A, Representative images of the time course of intracellular ROS accumulation at 5, 15, and 30 minutes after exposure to H$_2$O$_2$ (500 μmol/L) with or without nifedipine (0.1 or 1.0 μmol/L). Oxidative stress is indicated as green fluorescent dye. B, Fluorescence intensity of EPCs treated with nifedipine after exposure to H$_2$O$_2$ (100 or 500 μmol/L). Nifedipine protected EPCs against oxidative stress generated by 500 μmol/L of H$_2$O$_2$. Data are shown as the means±SEMs of triplicate experiments.
ment the migratory activity of EPCs in response to VEGF in the presence of H$_2$O$_2$ (Figure 7). Thus, nifedipine significantly rescued the impaired migratory function of EPCs under oxidative stress.

Discussion

Endothelial dysfunction is an initial trigger of atherosclerosis and a well-known predictor of future adverse cardiovascular events. Therefore, maintenance of endothelial function is
of primary importance in the management of patients with hypertension. Recent studies have shown that the number of CPCs is a significant predictor of future cardiovascular events.\textsuperscript{16,24} Release of progenitor cells from the bone marrow into the circulation has been shown to be endothelial NO synthase–derived NO dependent.\textsuperscript{25} In agreement with these reports, the extent of improvement in NO-dependent vasodilation (FMD difference) was correlated with the increase of CPCs in the present study. Although an improvement in the bioavailability of NO may also be related to the mild reduction of BP in the nifedipine group, it should be noted that nifedipine has the capacity to increase NO in stage 1 hypertensive subjects and in cultured endothelial cells.\textsuperscript{4,8,9} Furthermore, nifedipine stimulates VEGF release from vascular smooth muscle cells,\textsuperscript{10,26} which may also have contributed to the increase of endothelial NO formation and progenitor cell mobilization.\textsuperscript{25,27} In this regard, the number of CPCs could predict endogenous NO activity and endothelial integrity. Recent experimental data showed that these cells also contribute to the restoration of endothelial function, which implies that they may reflect the regenerative potential of the vasculature as well.\textsuperscript{28,29}

Our study also showed that nifedipine promoted differentiation and/or proliferation of cultured EPCs expanded from peripheral blood mononuclear cells (PB-MNCs). The number of these cells isolated from PB-MNCs is associated with endothelial function and the Framingham risk score.\textsuperscript{15} The precise origin of these cells is currently a subject of debate, but recent evidence suggests that the cells are of myeloid origin rather than being true progenitors.\textsuperscript{30,31} Nevertheless, these cells have been used clinically to induce angiogenesis and to repair vascular damage, indicating that their number in the circulation and angiogenic activity could still be used as indicators of vascular integrity.\textsuperscript{32} Taken together, these findings suggest that nifedipine improves endothelial function at least partly through modulation of proliferation and angiogenic activity of CPCs.

We found that nifedipine-treated EPCs showed a greater resistance to H2O2-mediated cellular oxidant stress, dysfunc-

and hypocitosis. Accumulating evidence suggests that an increase in ROS, such as H2O2 or superoxide anions (O2\textsuperscript{−}), is considerably involved in endothelial dysfunction in hypertension.\textsuperscript{33} O2\textsuperscript{−} and H2O2 are produced in vascular cells by multiple enzymatic systems, including vascular reduced nicotinamide-adenine dinucleotide phosphate oxidase, mitochondria, xanthine oxidase, and uncoupled endothelial NO synthase.\textsuperscript{34} Although some O2\textsuperscript{−} spontaneously degrades by reacting with NO, the O2\textsuperscript{−} signal is preserved by dismutation into H2O2, which has a prolonged and powerful oxidizing capability. This may explain why direct scavenging of H2O2 but not O2\textsuperscript{−} is more effective in vascular protection induced by ROS.\textsuperscript{35} Therefore, we hypothesized that H2O2 may be presumably responsible for the mechanism underlying the endothelial dysfunction in our study and investigated whether nifedipine directly ameliorates H2O2-induced oxidative stress in vitro. Vascular reduced nicotinamide-adenine dinucleotide phosphate oxidase has been reported as one of the primary sources generating H2O2.\textsuperscript{34} Yamagishi et al\textsuperscript{22,36} demonstrated that nifedipine directly abrogated vascular reduced nicotinamide-adenine dinucleotide phosphate oxidase activity in endothelial cells. On the other hand, it has been demonstrated that the expression level of antioxidant enzymes is remarkably higher in EPCs compared with mature endothelial cells, which contributes to endogenous tolerance against oxidative stress.\textsuperscript{37} Accordingly, we evaluated the effects of nifedipine on changes in the protein expression levels of H2O2-scavenging enzymes, i.e., catalase and glutathione peroxidase 1, as well as that of superoxide-dismutating enzymes in our EPCs. However, nifedipine had no effects on these expression levels (data not shown). Taking our results and the results of Yamagishi et al\textsuperscript{22,36} together, this suggests that nifedipine may exert its antioxidant and corresponding effects on EPCs through attenuation of reduced nicotinamide-adenine dinucleotide phosphate oxidase activity in EPCs as in the endothelium.\textsuperscript{38}

There are several limitations in the present clinical study. First, the number of study patients is small. Nevertheless, the nifedipine group showed a significant increase in CPCs and a strong positive correlation between the percentage of increase in the number of CPCs and the percentage of increase in endothelial function, suggesting that the action of nifedipine on vascular endothelium and CPCs may be potent. Second, the study was lacking a placebo group, and the placebo effects cannot be excluded. However, our preliminary observation on the direct effect of nifedipine on EPCs in a hypertensive rat model, i.e., the spontaneously hypertensive rat, demonstrates that the EPC number of the spontaneously hypertensive rat was significantly reduced, and, of note, nifedipine directly ameliorated the EPC number in a hypertensive model and not in the vehicle group (data not shown). These data suggest that the vascular protective effects of nifedipine observed in our clinical study presumably stem from its direct action on EPCs, as well as on the endothelium.

In conclusion, we demonstrated for the first time that nifedipine improves endothelial function, the number of CPCs in vivo, and the angiogenic activities of culture-expanded EPCs in vitro. These effects seem to be, at least in part, mediated by the maintenance of vascular integrity and improved resistance to oxidative stress.

**Perspectives**

To date, little information is available regarding the effects of antihypertensive agents on EPC biology. Clinical studies are also lacking to correlate the endothelial function and EPC kinetics under any pharmacological intervention.

The present study showed that nifedipine increased FMD and the numbers of CPCs and EPCs, along with a decrease in MDA-LDL levels. Improvement of FMD was positively correlated with an increase in CPCs. Moreover, nifedipine improved angiogenesis-related functions of EPCs in vitro, such as differentiation, migration, and resistance to oxidative stress. Therefore, nifedipine would have the ability to preserve endothelial integrity in patients with mild hypertension, at least in part by enhancing EPC numbers and functions.

To the best of our knowledge, this study is the first for any antihypertensive drugs to demonstrate the improvement of
endothelial function through the enhancement of function and circulating number of EPCs. Our findings provide a rationale for clinical observations, such as the International Nifedipine GITS Study: Intervention as a Goal in Hypertension Treatment, where nifedipine in comparison with a diuretic significantly reduced atherosclerosis progression, and also suggested that the number of CPCs and activity of EPCs could be used as predictors of the progression of vascular disease.39

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Disclosures
None.

References


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