Endothelial Progenitor Cells Stimulate Cerebrovascular Production of Prostacyclin By Paracrine Activation of Cyclooxygenase-2

Anantha Vijay R. Santhanam, Leslie A. Smith, Tongrong He, Karl A. Nath, Zvonimir S. Katusic

Abstract—In the present study we hypothesized that endothelial progenitor cells (EPCs) enhance production of vasoprotective substances in cerebral arteries. Isolated mononuclear cells from rabbit peripheral blood were cultured in endothelial growth medium (EGM-2) for 7 days to yield EPCs. Rabbit basilar arteries were exposed to autologous EPCs (≈5×10^5 cells) in vitro or in vivo. Twenty-four hours after intracisternal delivery of autologous EPCs, basilar arteries were isolated and expression of vasoregulatory proteins, production of prostacyclin (PGI₂), and cAMP were determined. Arteries transplanted with EPCs demonstrated increased protein expression of cyclooxygenase-2 and PGI₂ in adventitia, media, and endothelium. Furthermore, production of PGI₂ and arterial content of cAMP, second messenger for PGI₂, were significantly augmented after transplantation of EPCs. In contrast, production of thromboxane A₂ was significantly reduced, whereas production of prostaglandin E₂ remained unchanged. The increased production of PGI₂ and arterial content of cAMP were inhibited only by a selective cyclooxygenase-2 inhibitor, NS-398. In vitro or in vivo treatment of basilar artery with conditioned media from EPCs also caused increase in cyclooxygenase-2 and PGI₂ synthase protein expression associated with elevation of cAMP. Our results suggest that in cerebral arteries, paracrine effect of EPCs promotes vasoprotection by increasing PGI₂ production and intracellular concentration of cAMP. This effect appears to be mediated by activation of arachidonic acid metabolism via stimulation of cyclooxygenase-2/PGI₂ synthase pathway. (Circ Res. 2007;100:1379-1388.)

Key Words: PGI₂ synthase ▪ vasodilatation ▪ cerebral vasospasm ▪ COX-2 inhibitors ▪ cell therapy

Previous studies have demonstrated that administration of endothelial progenitor cells (EPCs) repairs the damaged endothelium and exerts reparative and regenerative effects in injured blood vessels.1-9 Although there is mounting experimental evidence for the involvement of EPCs in angiogenesis and vascular repair, the exact mechanism(s) underlying EPC-mediated vascular protection is not clearly understood. Prior studies from our group suggest that the resistance of EPCs to oxidative stress as well as their ability to release proangiogenic cytokines contributes to the beneficial effect of EPCs.10-12

Intravenous administration of bone marrow-derived progenitor cells improves neurological outcome during experimental stroke.13,14 The correlation of the number of circulating CD34-positive (hematopoietic progenitor) cells to the resilience of cerebral circulation to ischemic stress in humans suggests that these protective effects are likely extended to human disease and may arise from growth/angiogenic factors released by progenitor cells.11 It is conceivable that the ability of EPCs to augment the biosynthesis of vasoactive substances in the vessel wall may also contribute to the regenerative function of EPCs. Paracrine effects of the cytokines released by EPCs have also been proposed as a mechanism underlying such vasoprotection.11,12

In the present study, we hypothesized that EPCs enhance the production of vasoprotective substances in the cerebral arterial wall. We present evidence that transplantation of autologous EPCs stimulate arachidonic acid metabolism via cyclooxygenase-2 (COX-2), leading to increased production of prostacyclin (PGI₂) and cAMP. Paracrine function of EPCs appears to be responsible for the observed stimulatory effect on vascular biosynthesis of PGI₂.

Materials and Methods

Animals
Male New Zealand white rabbits (2 to 3 kg) were used in this study. Rabbits were anesthetized with intramuscular injection composed of ketamine (35 mg/kg), xylazine (5 mg/kg), and acepromazine (2.3 mg/kg). Animals were anesthetized and euthanized with intravenous Sleepaway (3 mL, Fort Dodge Animal Health). All procedures were approved by the Institutional Animal Care and Use Committee of the Mayo Clinic College of Medicine.

Original received August 22, 2006; resubmission received December 28, 2006; revised resubmission received February 21, 2007; accepted March 21, 2007.

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Circulation Research is available at http://circres.ahajournals.org

DOI: 10.1161/01.RES.0000265848.55035.5d
Isolation and Culture of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells were isolated from rabbit peripheral blood by density gradient centrifugation with Ficoll-Paque Plus (GE Healthcare) and cultured for 7 days in endothelial growth medium-2 (EGM-2) (Cambrex Corp) as described earlier.11 Medium was changed daily and after culturing for 7 days, the adherent cells (so-called “early EPCs” or11,16,17 were harvested. In some experiments, mononuclear cells (day 0, 5×10⁵ cells) were seeded on fibronectin-coated tissue culture plates for 24 hours and used as controls.

Incubation of Basilar Arteries With EPCs In Vitro

Rabbit basilar arteries were rinsed with Krebs–Ringers solution18 (composition [in mmol/L]: NaCl 118.3, KCl 4.7, CaCl₂ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, calcium-ethylenediaminetetraacetic acid 0.026, and glucose 11.1, 37°C, pH 7.4) and exposed to autologous EPCs (∼5×10⁴ cells) in 1 well (3 mL volume) of a 6-well tissue culture plate for 24 hours in EGM-2 at 37°C. Overnight incubation of arteries in EGM-2 alone did not affect vasomotor function (data not shown).

In Vivo Administration of EPCs

A 25-gauge needle was injected aseptically into the cisterna magna, and 300 μL of cerebrospinal fluid (CSF) was aspirated and replaced with EGM-2 alone (vehicle) or EGM-2 containing 5×10⁴ EPCs.19,20 Twenty-four hours after injection, rabbits were euthanized and the basilar arteries were isolated.

Paracrine Effects of EPCs

On day 7 of culture of EPCs, the media in which cells were grown for 24 hours were collected and used as conditioned media. Isolated basilar arteries were incubated in vitro for 24 hours at 37°C in this conditioned media. For in vivo studies, conditioned media from day 7 EPCs were concentrated using Centriprep Centrifugal Filter Units (Millipore). Briefly, ∼6 mL of conditioned media was added to Centriprep tube and centrifuged at 3000 rpm at 4°C for 90 minutes. The filtrate was discarded, and the supernatant containing the concentrated conditioned media (∼600 μL) was collected. EGM-2 was centrifuged in parallel, as described above, and used for controls. CSF (600 μL) was aspirated and replaced with concentrated conditioned media or EGM-2 (600 μL) as described earlier. Twenty-four hours later, the rabbits were euthanized and the basilar arteries were isolated.

Western Blot

Soluble proteins were extracted by mincing and homogenizing basilar arteries in lysis buffer, as described earlier.20,21 Blots were incubated with monoclonal antibodies (1:500 dilution) against COX-1 (Cayman), COX-2 (BD Transduction), inducible NO synthase (iNOS) (BD Transduction), actin (1:2000; Santa Cruz Biotech), polyclonal antibodies against endothelial NO synthase (eNOS) (BD Transduction), and PGI₂ synthase (Cayman).

Immunohistochemical Analysis

Basilar arteries were embedded in paraffin, and 5-μm sections were cut. Staining was performed on deparaffinized sections. Endogenous peroxidases were blocked with 20 minutes of incubation in 0.3% H₂O₂ in methanol. Nonspecific binding was blocked by incubation of tissue with diluted goat serum for 20 minutes. Sections were then incubated with monoclonal anti-COX-2 antibody (1:100; BD Transduction) for 2 hours at room temperature. Secondary antibody from Vectastain Elite ABC Kit (Vector Laboratories) was applied to sections for 1 hour at room temperature. For visualization, sections were incubated with DAB substrate (Vector Laboratories) for 10 seconds and counterstained with hematoxylin. Mouse IgG was used as negative controls.

Analysis of Vascular Reactivity

Isolated arteries were connected to a force transducer for recording of isometric force and placed in organ baths filled with 25 mL Krebs solution (37°C; 94% O₂/6% CO₂; pH 7.4).21 Concentration-dependent response curves to acetylcholine, and diethylammonium (Z)-1-(N,N-diethylamino)diazen-1-IM1,2-diolate (DEA-NONOate) were cumulatively obtained during submaximal contractions to histamine.

Measurement of PGI₂, Thromboxane A₂, and Prostaglandin E₂

Basilar arteries isolated from rabbits injected either with EGM-2 or EPCs were incubated in Krebs solution in a CO₂ incubator at 37°C.
for 30 minutes. After incubation, the arteries were dried and weighed, whereas the Krebs solution was used for determination of 6-keto prostaglandin F$_1$ ($\text{PGF}_1$), TxB$_2$ (stable metabolite of thromboxane A$_2$ [TxA$_2$]), and prostaglandin E$_2$ (PGE$_2$) by their respective enzyme immunoassay kits (Cayman Chemical, Ann Arbor, Mich). In some experiments, basilar arteries were incubated with either SC-560 (10$^{-6}$ mol/L) or NS-398 (10$^{-5}$ mol/L) for 30 minutes at 37°C. After incubation, 6-keto prostaglandin F$_1$ was measured as described above.

**Measurement of cAMP**

Radioimmunoassay kits (Amersham) were used to perform the measurements as described elsewhere.$^{20,21}$ In some experiments, basilar arteries were incubated with either SC-560 (10$^{-6}$ mol/L) or NS-398 (10$^{-5}$ mol/L) for 30 minutes at 37°C. After incubation, 6-keto prostaglandin F$_1$ was measured as described above.

**Drugs**

DEA-NONOate was obtained from Cayman Chemical. All other drugs used in the study were obtained from Sigma. The concentration of all drugs is expressed as the final moles per liter.

**Statistical Analysis**

Results of the study are expressed as means±SEM for n (the number of rabbits in each group) animals used in each experimental group. Relaxations are expressed as percentage of maximal relaxation to 3×10$^{-4}$ mol/L papaverine. cAMP values across different groups were assessed by 1-way ANOVA, followed by pairwise comparisons. Differences among 6-keto prostaglandin F$_1$ values, TxB$_2$, PGE$_2$, and densitometric comparisons were compared by Student’s t test. Differences among relaxation values across concentration–response curves were analyzed by 2-way repeated-measures ANOVA, followed by Student–Newman–Keuls post hoc test. A probability value of <0.05 was considered statistically significant.

**Results**

**Characterization of Early EPCs**

Culturing mononuclear cells for 7 days in EGM-2 yielded early EPCs, which expressed the hematopoietic marker CD34, as well as endothelial markers CD31, and the vascular endothelial growth factor receptor-2 (VEGFR-2) Flk-1 (Figure 1). As reported previously by our group, early rabbit EPCs incorporated acetylated LDL

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and demonstrated isolectin binding (data not shown). Further culture (≈4 weeks) gave rise to colonies of out-growth cells (late EPCs) exhibiting cobblestone morphology and monolayer growth pattern typical of endothelial cells at confluence.

In Vitro Effect of EPCs on Rabbit Basilar Arteries

Contraction to histamine was reduced in basilar arteries incubated with EPCs (Figure 2a) in comparison with those arteries incubated in EGM-2. In contrast, contraction to
histamine remained unaltered in arteries incubated with mononuclear cells (Figure 2a). In the presence of a selective COX-2 inhibitor, NS-398, contractions to histamine were augmented in arteries treated with EPCs (Figure 2b), thereby suggesting that upregulation of COX-2 is responsible for the inhibitory effect of EPCs on the contractile reactivity to histamine. The maximal contraction to KCl (80 mmol/L) remained unaffected in all 3 groups studied (data not shown). Acetylcholine-induced relaxation was significantly potentiated in arteries exposed to EPCs (Figure 2c), whereas the maximal relaxation to acetylcholine in arteries exposed to mononuclear cells were significantly attenuated (Figure 2c). Treatment with a COX-2 inhibitor, NS-398, abolished enhancement of the acetylcholine effect in arteries treated with EPCs (Figure 2d). Western blotting studies on arteries incubated with EPCs in vitro demonstrated significantly increased protein expression of COX-2 and PGI2 synthase (Figure 3a) in comparison with arteries treated with EGM-2. In agreement, arterial content of cAMP, the second messenger of PGI2 was significantly elevated in arteries exposed to EPCs in comparison with those incubated with EGM-2 (Figure 3b). Mononuclear cells did not affect either expression of PGI2 synthase (data not shown) or the levels of cAMP (Figure 3b). To determine whether the effect of EPCs is dependent on their paracrine function, isolated basilar arteries were incubated for 24 hours in vitro in conditioned media obtained from EPCs. As illustrated in Figure 3c, expression of COX-2 and PGI2 synthase was selectively increased in arteries incubated in conditioned media.

**Intracisternal Administration of EPCs In Vivo**

Intracisternal administration of EPCs into rabbits in vivo also increased protein expression of COX-2 and PGI2 synthase in basilar arteries (Figure 4). Delivery of EPCs into cisterna magna of rabbits did not affect the expression of either the constitutive or inducible isoform of NO synthase (Figure 4). Production of PGI2 was significantly increased in basilar arteries treated with EPCs (Figure 5a). Increased PGI2 production in PGI2-treated arteries was sensitive to the COX-2 inhibitor NS-398 (Figure 5a), whereas the COX-1 inhibitor SC-560 (Figure 5a) did not affect the increased PGI2 production. In contrast to PGI2 production, production of TXA2 was significantly reduced in arteries treated with EPCs (Figure 5b), whereas production of PGF2 was not affected (Figure 5c). Furthermore, cAMP levels were also significantly increased in arteries of rabbits injected with EPCs in comparison with EGM-2-treated arteries (Figure 5d). Treatment of the basilar arteries with a nonselective COX inhibitor, indomethacin or a selective COX-2 inhibitor, NS-398, inhibited the increase in cAMP levels (Figure 5d), whereas treatment with a selective COX-1 inhibitor, SC-560, did not affect cAMP (Figure 5d). Immunohistochemical staining of sections of EPC-treated arteries showed the presence of COX-2 in adventitia, media, and the endothelium (Figure 6). Concomitantly with increased expression of COX-2, PGI2 synthase expression was also higher (data not shown).

In contrast to in vitro findings, contractions to histamine and relaxations to acetylcholine or DEA-NONOate were not affected by in vivo delivery of EPCs (Table). In this regard, it is important to point out that levels of cAMP were 4- to
5-fold lower in vivo (Figure 5d) as compared with values detected in vitro (Figure 3b), thereby suggesting that EPC-induced elevation of cAMP in vivo was not sufficient to alter vasomotor function.

**In Vivo Paracrine Effects of EPCs**

In vivo injection of conditioned media from EPCs into the cisterna magna also selectively increased protein expression of COX-2 and PGI2 synthase (Figure 7a). In agreement, arterial content of cAMP from rabbits injected with conditioned media from EPCs was significantly higher as compared with arteries of rabbits injected with EGM-2 (Figure 7b).

**Discussion**

This is the first study to examine the effects of EPCs on vascular function of cerebral arteries. We report several novel findings. First, we provide evidence that EPCs cause elevation of COX-2 and PGI2 synthase protein expression in the cerebral arterial wall, whereas expression of COX-1, eNOS, and iNOS were not affected. Second, local production of vasoprotective substances, PGI2 and its second messenger cAMP, were significantly increased in arteries treated with EPCs. Third, EPCs reduced production of TXA2 in the cerebral arterial wall but did not affect biosynthesis of PGE2, demonstrating a unique profile of prostanoids released from cerebral arterial wall stimulated by EPCs. Fourth, selective pharmacological inactivation of COX-2 enzymatic activity abolished the stimulatory effect of EPCs on PGI2 production as well as elevation of cAMP. Finally, conditioned media obtained from EPCs selectively elevated expression of COX-2 and PGI2 synthase and the arterial content of cAMP, suggesting that paracrine function of EPCs is responsible for the observed vascular effects. We wish to point out that the effects of EPCs were consistent under in vitro and in vivo experimental conditions, thereby strongly supporting our conclusion that EPCs promote activation of arachidonic acid metabolism via the COX-2/PGI2 pathway.

Numerous prior studies, including those from our group, have demonstrated that transplantation of EPCs stimulates endothelial repair and the recovery of endothelial function after vascular injury.5–9,11 The exact mechanisms responsible for the effects of EPCs are unknown but are mediated, most likely, by production and release of proangiogenic cyto-
In the present study, we first used an in vitro approach to determine the effect of EPCs in isolated rabbit basilar artery. We also examined the mechanisms activated by EPCs in the arterial wall as well as resulting changes in vascular reactivity. Because EPCs are isolated from a nuclear population of circulating cells, we used exposure to mononuclear cells as a negative control to demonstrate selectivity of the effect of EPCs. EPCs inhibited contraction to histamine and enhanced relaxation to acetylcholine. In contrast, treatment with mononuclear cells did not affect the contractile response to histamine whereas maximal relaxation to acetylcholine was reduced. These observations ruled out nonselective effect of cytokines released from cultured circulating cells and demonstrated the selective ability of EPCs or cytokines released from EPCs to affect biochemical and functional characteristics of cerebral arteries. Further analysis suggested that the upregulation of COX-2/PGI2 pathway and subsequent stimulation of adenylate cyclase in the arterial wall may explain the observed effects of EPCs on vasomotor function. Detected high content of cAMP in arteries exposed to EPCs provided a plausible explanation for the reduced reactivity to vasoconstrictor histamine. Enhancement of relaxation to acetylcholine is also best explained by high content of PGI2/cAMP in the cerebral arterial wall. PGI2 may augment relaxations to NO released by acetylcholine. Indeed, previous studies demonstrated that PGI2 and NO have synergistic biological effects. Potentiation of acetylcholine-induced relaxation was sensitive to COX-2 inhibition by NS-398, thus confirming the involvement of COX-2 in this effect. Consistent with in vitro findings, activation of COX-2/PGI2/cAMP signal transduction pathway in the arterial wall emerged as a key mechanism responsible for the effect of EPCs in vivo. Most notably, production of PGI2 and its second messenger, cAMP, however, we wish to point out that in vivo, arterial cAMP levels were ~4- to 5-fold lower as compared with levels detected in vitro. Although we do not have an exact explanation for this difference, it is most likely caused by constant circulation of cerebrospinal fluid in vivo and subsequent decrease in perivascular concentration of substances released from EPCs. This may reduce the concentration gradient required for diffusion of paracrine substances through vascular wall. In contrast, incubation of arteries for 24 hours with EPCs in vitro is associated with accumulation and gradual increase in release of paracrine substances. Therefore, it is possible that the observed increase in vasodilatory effect on biosynthesis of PGI2. In agreement with the in vitro findings, activation of COX-2/PGI2/cAMP signal transduction pathway in the arterial wall may explain the observed effects of EPCs on vasomotor function.

### Vasomotor Studies of Basilar Arteries From Rabbits Injected Intracisternally With EGM-2 or EPCs

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>−Log EC50</th>
<th>Maximal Response (%)</th>
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<tbody>
<tr>
<td>Constrictions to histamine</td>
<td></td>
<td></td>
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<tr>
<td>EGM-2</td>
<td>6.06±0.14</td>
<td>100</td>
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<tr>
<td>EPCs</td>
<td>6.02±0.10</td>
<td>100</td>
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<tr>
<td>Relaxations to acetylcholine</td>
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<tr>
<td>EGM-2</td>
<td>5.86±0.11</td>
<td>79.07±4.74</td>
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<tr>
<td>EPCs</td>
<td>6.09±0.21</td>
<td>86.51±4.15</td>
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<tr>
<td>Relaxations to DEA-NONOate</td>
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<tr>
<td>EGM-2</td>
<td>5.79±0.11</td>
<td>80.42±4.16</td>
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<tr>
<td>EPCs</td>
<td>6.75±0.08</td>
<td>100</td>
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<tr>
<td>Contractions to histamine (10−8 to 10−5 mol/L) in EGM-2– and EPC-treated arteries; 100%=2.09±0.17 g and 100%=2.22±0.13 g (n=6), respectively. Relaxations to acetylcholine and DEA-NONOate are expressed as the percentage of maximal relaxations to 3×10−4 mol/L papaverine. Relaxation responses to acetylcholine (10−8 to 10−5 mol/L) and DEA-NONOate (10−8 to 10−5 mol/L) were obtained during histamine-induced contractions (EC50). Relaxations to acetylcholine in EGM-2– and EPC-treated arteries; 100%=1.32±0.17 g (EGM-2), 100%=1.31±0.09 g (EGM-2+NS-398), 1.01±0.13g (EPCs), and 1.44±0.27g (EPCs+NS-398) (n=6), respectively. Relaxations to DEA-NONOate in EGM-2 and EPCs-treated arteries; 100%=1.14±0.13 g and 100%=1.03±0.14 g (n=6), respectively. Relaxation responses among multiple groups were compared by 2-way repeated-measures ANOVA.</td>
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concentration of substances released from EPCs. Morphological analysis demonstrated that increased expression of COX-2 protein was present throughout basilar artery, including adventitia, media, and endothelium, thus adding support to the contention that in vivo diffusion of EPC-derived substances from the perivascular space is required for observed upregulation of COX-2 and PGI₂ synthase. Consistent with this interpretation, we detected comparatively moderate increase in arterial cAMP in vivo. This may also explain the inability of intracisternal cell delivery to significantly affect vasomotor function. Evidently, further improvement of cell-delivery techniques and cell targeting to cerebral arteries of interest will be required to achieve a detectable effect on reactivity of isolated arteries.

The protective effects of COX-2 and PGI₂ on the vasculature are well established. Expression of COX-2 and biosynthesis of PGI₂ are upregulated by elevation of shear stress, a key regulator of vascular homeostasis. COX-2 is a major source of PGI₂ in humans and a potent endogenous vasodilator and anti-atherogenic molecule. PGI₂ prevents platelet aggregation, inhibits smooth muscle proliferation, and stimulates angiogenesis. The importance of PGI₂ biosynthesis for regenerative function of EPCs is not clear at the present time. However, a recent study by You et al proposed that the vasodilator effect of EPCs is an essential component of their proangiogenic effect. Given the fact that PGI₂ is a potent vasodilator and exerts proangiogenic activity, it is very likely that stimulation of PGI₂ biosynthesis in the arterial wall is a key component of vascular repair induced by EPCs. Indeed, EPCs suppressed production of TxA₂ and did not affect production of PGE₂, suggesting that vasoprotective PGI₂ is the most abundant prostanoid released from arterial wall activated by EPCs. We also wish to point out that expression of COX-1, eNOS, and iNOS was not affected in cerebral arteries treated with EPCs in vivo. These observations demonstrate selectivity of the effects of EPCs for enzymatic activities of COX-2 and PGI₂ synthase.

In our previous study, we reported that rabbit EPCs produce and release proangiogenic cytokines and we proposed that the paracrine effect of EPCs is responsible for the
beneficial effect of EPCs on repair of injured endothelium.\textsuperscript{11} Gneechi et al.\textsuperscript{10} also demonstrated that the cytoprotective effect of mesenchymal cells is mediated by their paracrine effect on ischemic myocardium. The results of the present study expand this concept to cerebral circulation and demonstrate that the effect of EPCs is dependent on production and release of EPC-derived vasoactive substances. Further studies are required for identification of their chemical nature; however, the most likely candidates are VEGF, transforming growth factor-β, and interleukin-8. All of these cytokines are released from the rabbit EPCs\textsuperscript{11} and are known stimulators of COX-2 and production of PGI\textsubscript{2}.\textsuperscript{40–44}

Pharmacological analysis of the effects of EPCs on cerebral arteries demonstrated that the inactivation of COX-2 (but not COX-1) by a selective COX-2 inhibitor, NS-398,\textsuperscript{45} abolished EPC-induced increase in PGI\textsubscript{2} production as well as increased cAMP content in arterial wall. We regard this observation as important because it offers a new insight into the mechanism of increased risk for cardiovascular disease associated with COX-2 inhibitors. Recently, the COX-2 inhibitor rofecoxib was withdrawn from the market because of an increased incidence of myocardial infarction and stroke detected during the course of clinical trials with this compound. The results of the present study are the first to suggest that activation of COX-2 in arterial wall by a paracrine effect of EPCs is an essential component of the EPC-induced vasoprotection. Although the extent to which the regenerative and reparative effects of EPCs is dependent on arachidonic acid metabolism is unclear, it seems likely that inhibition of COX-2 may impair the regenerative function of EPCs, thereby contributing to the adverse cerebrovascular effects of COX-2 inhibitors.

The inductive effect of EPCs on vascular expression of COX-2, production of PGI\textsubscript{2}, and vasodilatory responses may not only be relevant to the regenerative effects of EPCs in pathologic states but may also be germane to physiologic conditions. Although current interest in EPCs centers predominantly on their role in angiogenesis and repairing injured blood vessels, the evidence is clear that EPCs are present and functionally active in healthy, disease-free conditions. The results of the present study lead us to speculate that EPCs, via their inductive effect on PGI\textsubscript{2}, may contribute, at least in part, to vascular protection in healthy, disease-free conditions.

The results reported in the present study support the concept that a COX-2/PGI\textsubscript{2}/cAMP vasoprotective pathway is activated in blood vessels exposed to EPCs. Our findings offer a novel insight into the molecular mechanism underlying interaction between EPCs and cerebral arterial wall. They provide support for the idea that in the cerebral arterial wall, the vasoprotective effects of EPCs are mediated by activation of arachidonic acid metabolism. If proven in human studies, this concept will have important implications for successful harnessing of the therapeutic effects of EPCs in the clinical arena.

Sources of Funding
This work was supported in part by National Heart, Lung, and Blood Institute grants HL-53524, HL-58080, and HL-66958 (to Z.S.K.), American Heart Association Postdoctoral Fellowship 06-20017Z-1 (to A.V.R.S.) and The Mayo Foundation.

Disclosures
None.

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Circ Res. 2007;100:1379-1388; originally published online March 29, 2007;
doi: 10.1161/01.RES.0000265848.55035.5d
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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