Endothelial dysfunction is a hallmark of cardiovascular and cerebrovascular disease. Restoring the endothelial lining to normal is critical to slowing or reversing the progression of vascular disease. The progression of endothelial regeneration following mechanical disruption and its effect on vascular function has been described in normal porcine coronary arteries in the absence of risk factors for vascular disease.\(^1\)\(^-\)\(^3\) Despite regrowth of endothelium in arteries shortly after denudation, the endothelium was morphologically different. Smooth muscle function remained normal but endothelium-dependent relaxation progressively worsened over several weeks. Although these early studies assumed that the regeneration of the endothelial lining was because of the migration and proliferation of neighboring cells, the proliferation rate of endothelial cells is low.\(^4\) We now know that alternative repair mechanisms contribute to the regrowth of endothelium.

Recent studies suggest that a damaged endothelial lining can be restored by endothelial progenitor cells (EPCs). Circulating EPCs derived from bone marrow contribute to the repopulation by seeding the intimal lining with new cells.\(^3\)\(^-\)\(^6\) These circulating EPCs home in on injured areas and accelerate the regeneration of new endothelium. In culture, EPCs acquire both morphological and functional characteristics of endothelial cells expressing von Willebrand factor, CD31, and KDR, a receptor for vascular endothelial growth factor (VEGF).\(^4\) They release nitric oxide, form tube-like structures in vitro and enhance neovascularization and blood flow in ischemic hindlimb in vivo.\(^7\) Thus EPCs provide a potential new approach to repair vascular injury and reverse the progression of vascular disease.

Although EPCs promote endothelial regeneration and angiogenesis, atherosclerosis is not limited to damage of the endothelial lining. Activation of an inflammatory response induces infiltration of monocytes and smooth muscle proliferation contributing to the development of atherosclerotic lesions.\(^4\) Risk factors including hyperlipidemia, hyperglycemia and aging promote the release of cytokines, reactive oxygen species (ROS) and other inflammatory mediators from endothelium, vascular muscle and infiltrating white cells that inactivate nitric oxide, further damaging the endothelium and the underlying vascular muscle and resulting in vascular dysfunction. The ability of EPCs to restore the health of the entire vessel wall and return vascular function to normal is unknown.

Katusic and coworkers have described a systematic series of studies to determine the potential of EPCs to regenerate endothelium and restore vascular function to normal in the cerebral circulation.\(^8\)\(^-\)\(^11\) The paracrine factor IL-8 released from cultured EPCs stimulated proliferation of mature endothelial cells from human umbilical vein and coronary artery.\(^10\) Transplantation of autologous EPCs to denuded carotid arteries increased endothelial regrowth and improved endothelium-dependent relaxation in the absence of other risk factors.\(^8\) However to provide an effective approach to the treatment of vascular disease EPCs must restore endothelial function in the setting of injury/inflammation-induced oxidative stress. In the presence of atherosclerosis ROS binds to nitric oxide (NO) to form peroxynitrite and decreases bioavailability of NO and vascular responses (Figure). EPCs may be resistant to oxidative stress, in part, because of a higher expression of MnSOD.\(^9\) Expression of copper zinc superoxide dismutase and catalase in EPCs was similar to mature endothelium. Thus EPCs may inhibit progression of vascular disease through their antioxidant effects to preserve NO and release of paracrine factors to stimulate endothelial growth.

The study by Santhanam et al in the current issue of Circulation Research further examined mechanisms involved in the repair process by comparing the effects of EPCs on vascular function following ex vivo and in vivo exposure.\(^11\) Twenty-four hours after exposure to autologous EPCs, levels of vasodilator prostacyclin and cAMP were increased whereas levels of vasoconstrictor thromboxane was reduced.\(^11\) The increase was associated with an increase in the expression cyclooxygenase-2 (COX-2) and prostacyclin (PGI2) synthase within all layers of the vascular wall including endothelium, smooth muscle and adventitia. However there were no differences in expression of cyclooxygenase-1, endothelial nitric oxide synthase (eNOS) or inducible NOS (iNOS). This increase in vasodilator arachidonic acid metabolites was associated with restoration of normal vascular function following in vitro administration but not in vivo. This differential effect of ex vivo and in vivo exposure on vascular function was attributed to a greater increase in COX-2 and PGI2 synthase with ex vivo exposure. EPC conditioned media also elevated COX-2 and PGI2 synthase. The specific paracrine factor(s) that induced COX-2 and PGI2 synthase was not identified but may include VEGF, trans-
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EPCs migrate to the site of endothelial damage and release an unidentified paracrine factor to upregulate the expression of COX-2 and increase levels of prostacyclin (PGI₂). PGI₂ activates adenylyl cyclase to increase levels of cAMP and produce vasodilation. Paracrine factors including VEGF, transforming growth factor-β (TGF-β), or interleukin-8 (IL-8) released from EPCs may upregulate COX-2. In the presence of risk factors for the development of vascular disease NO is inactivated by elevated levels of superoxide anion (O₂⁻) forming peroxynitrite (ONOO⁻) and reducing vasodilation. EPCs contain elevated levels of manganese superoxide dismutase (MnSOD) which dismutates superoxide anion to preserve NO function. Despite evidence that human-derived EPCs contain endothelial NOS and release nitric oxide (NO), short-term exposure of injured arteries to EPC did not increase the expression of either endothelial or inducible NOS.

formulating growth factor-β, or interleukin-8. All are released from EPCs and stimulate COX-2 and PGI₂ production. This study demonstrated that at least in vitro EPCs can restore vascular function but the benefits of in vivo transplantation of EPCs on vascular function have yet to be demonstrated (Figure).

It is somewhat surprising that there was no change in expression of eNOS or NO-mediated vasodilation in rabbit carotid arteries exposed to EPCs. Human EPCs express eNOS and with appropriate stimulation release NO. Is this difference because of differences in species, time course, or presence of risk factors that alter expression of NOS in attached EPCs? Bone marrow derived cells from eNOS deficient mice are less effective than bone marrow derived from wild type mice in neovascularization of ischemic hindlimbs.4 Because NO bioavailability is reduced in the presence of cardiovascular disease the effectiveness of EPCs maybe reduced. Thus simply injecting EPCs into a hostile environment with decreased levels of NO may not produce the desired long-term benefits.

But what happens when EPCs are seeded on unfertile ground? Several factors contribute to the success of transplanted EPCs to restore endothelium. EPCs differentiate into endothelium when exposed to the appropriate growth factors such as VEGF. In contrast, a change in the environment to include macrophage-colony stimulating factor or interleukin-4 with granulocyte-macrophage-colony stimulating factor redirects the differentiation of EPCs to macrophages or dendritic cells.4 The number of EPCs declines with age and in individuals with risk factors for cardiovascular disease such as smoking, diabetes and aging.4 EPCs derived from younger apoE animals were more effective in delaying the progression of atherosclerosis in aorta of apoE deficient mice.12 But EPCs were injected every 2 weeks for 10 weeks. Would a single injection of EPCs from wild-type mice have been sufficient to alter the progression of disease?

Although the results of the study by Santhanam et al11 contribute to our understanding of the EPCs in the recovery of endothelial function with mechanical disruption, many questions regarding the potential benefits of EPCs in the treatment of vascular disease remain. Can EPCs be administered to promote the generation of new endothelium in the presence of atherosclerosis, diabetes or hypertension that will restore vascular function? And if function is improved how long will the benefits persist? Will the functional benefits persist in the absence of an improvement in NO bioavailability? Repopulation of the endothelial lining with EPCs may initially restore vascular function to normal, but without alterations in the local environment will the changes be permanent? Studies to date have focused on mechanical injury of normal arteries. The local environment is an major determinant in the fate of circulating EPCs.4 Risk factors such as aging, ischemia, and oxidized LDL interfere with EPC mobilization, production of chemoattractant factors, and survival/differentiation of EPCs. Without an alteration in these risk factors, addition of EPCs may actually promote the development of atherosclerosis. Before administration of EPCs can be used as a therapeutic approach these and many other questions will need to be addressed.

“A sower went out to sow his seed. Some fell by the wayside, and it was trampled down, and the birds devoured it. Some fell on rock, and it withered away because it lacked moisture. And some fell among thorns and the thorns sprang up and choked it. But others fell on good ground, sprang forth and yielded a crop a hundredfold” (Luke 8:5 to 8).

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References


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