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Endothelial Progenitor Cells for Cardiovascular Regeneration

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Abstract

Endothelial progenitor cells (EPCs) are peripheral blood mononuclear cells that can differentiate into mature endothelial cells. Adult EPCs were first discovered in human peripheral blood in 1997. Since then, the potency of EPCs for cardiovascular regeneration has been demonstrated in several preclinical studies, and investigators are beginning to evaluate the therapeutic utility of EPCs in early-phase clinical trials. This review summarizes the progression of basic, preclinical, and clinical research into the potential use of EPC therapy for cardiovascular regeneration.

Introduction

Endothelial progenitor cells (EPCs) are immature cells capable of differentiating into mature endothelial cells. In 1997, EPCs were first identified in adult human peripheral blood as CD34 antigen-positive (CD34+) mononuclear cells (MNCs) (Asahara et al. 1997). Since this discovery, the pathophysiological role and therapeutic application of adult (i.e., postnatal) EPCs have been the subjects of intense experimental and clinical investigation. This review summarizes the progress of basic, preclinical, and clinical research into this novel therapeutic modality.

Characteristics of Postnatal EPCs

Endothelial progenitor cells and hematopoietic stem cells (HSCs) share many surface marker antigens, such as Flk-1, Tie-2, c-Kit, Sca-1, AC133, and CD34 (Flamme and Risau 1992, His 1900, Weiss and Orkin 1996), and likely descend from a common precursor cell (hemangioblasts) during embryonic development. In 1997, Asahara et al. isolated CD34+ MNCs from human peripheral blood, cultured them on fibronectin, then tested them for the expression of leukocyte (CD45) and endothelial cell (CD34, CD31, Flk-1, Tie-2, E selectin, and endothelial nitric oxide synthase [eNOS]) markers to confirm that the cultured cells developed an EC-like phenotype. After intravenous injection into immunodeficient mice with experimental hindlimb ischemia, fluorescent dye-labeled CD34+ cells were incorporated into the sites of ischemic neovascularization. On the basis of this compelling evidence, the isolated

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CD34+ MNCs were identified as postnatal EPCs. Since the publication of this initial report, several groups have used a variety of methodologies to identify EPCs (Gehling et al. 2000, Gunsilius et al. 2000, Lin et al. 2000, Peichev et al. 2000, Shi et al. 1998).

Like HSCs, adult EPCs are believed to originate from the bone marrow; thus, transplantation of bone marrow containing labeled EPCs enables cells descended from the bone marrow to be identified after incorporation into various tissues. Bone marrow-derived EPCs have been found in foci of neovascularization during wound healing and tumor growth, in ischemic skeletal and cardiac muscle, and in corneas after corneal micropocket surgery. Similar EPC incorporation has been observed during endometrial neovascularization after induced ovulation or estrogen administration (Asahara et al. 1999a). These findings suggest that the bone marrow is a major source of EPCs and that endogenous mobilization of EPCs from the bone marrow into the peripheral blood occurs in response to a physiological need for neovascularization. The mobilized EPCs are then recruited into the foci of neovascularization and contribute to new blood vessel formation (Figure 1). This mechanism is also supported by clinical observations of EPC mobilization in patients with burns (Gill et al. 2001) or acute myocardial infarction (Shintani et al. 2001) and in patients undergoing coronary artery bypass graft surgery.

Therapeutic Modulation of EPCs

Techniques that influence EPC mobilization and incorporation could enhance EPC therapy both directly, by improving neovascularization, and indirectly, by enabling more efficient harvesting of EPCs from peripheral blood. The mobilization and incorporation of EPCs can be modulated by recombinant pharmaceuticals and natural hematopoietic or angiogenic stimulants.

Granulocyte macrophage-colony stimulating factor (GM-CSF) induces colony formation in several cell types (e.g., hematopoietic progenitor cells, myeloid lineage cells, bone-marrow stromal cells) and also enhances both the neovascularization of severely ischemic tissues and the *de novo* vascularization of corneas after corneal micropocket surgery. The observed enhancement is believed to evolve from amplified EPC mobilization and increased recruitment of circulating EPCs into the site of new blood vessel formation (Takahashi et al. 1999). In a pilot clinical trial, patients with acute myocardial infarction displayed a significant increase in EPC mobilization after GM-CSF injection (Seiler et al. 2001).

Vascular endothelial growth factor (VEGF) and granulocyte-colony stimulating factor (G-CSF) (Gehling et al. 2000) are also potent stimulators of EPC mobilization. VEGF is perhaps the most critical growth factor for vasculogenesis and angiogenesis (Carmeliet et al. 1996, Ferrara et al. 1996, Shalaby et al. 1995). The effect of VEGF on EPCs has been observed in mice after intraperitoneal injection of recombinant human VEGF₁₆₅ (Asahara et al. 1999b) and in patients with critical limb ischemia (Kalka et al. 2000a) and myocardial ischemia (Kalka et al. 2000c) who were treated with VEGF gene transfer therapy. Bone marrow-derived EPCs were efficiently mobilized into the circulation by G-CSF injection during placebo-controlled clinical trials in patients who underwent successful primary coronary interventions (Ripa et al. 2006, Zohnhofer et al. 2006).

Stromal cell-derived factor-1 (SDF-1), which plays an important role in trafficking HSCs, contributes to neovascularization by enhancing the mobilization, recruitment, and proliferation of EPCs (Peichev et al. 2000, Yamaguchi et al. 2003). Statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors that catalyze a rate-limiting step in cholesterol biosynthesis, enhance EPC mobilization by stimulating the Akt signaling pathway (Dimmeler et al. 2001, Llevadot et al. 2001, Vasa et al. 2001).

EPC Therapy for Ischemic Diseases: Preclinical Studies

Tissue ischemia caused by peripheral, cerebral, and coronary artery disease can be attenuated by the development of collateral circulation, so after the role of EPCs in neovascularization was recognized, investigators began to evaluate the potential therapeutic impact of EPC administration for treatment of ischemic conditions. Kalka et al. (2000b) intravenously administered *ex vivo* expanded EPCs obtained from human peripheral blood into immunodeficient mice with hindlimb ischemia. Recovery of blood flow was greater in mice treated with EPCs than in control mice and mice that received mature endothelial cells, and histological examinations confirmed EPC incorporation and differentiation into endothelial cells. Limb salvage ratio, perhaps the most important endpoint in critical limb ischemia, was also dramatically improved with EPC treatment. Murohara (2001) reported similar benefits with the administration of EPCs isolated from cord blood in a nude rat model of hindlimb ischemia. Experiments performed in our lab evaluated EPC therapy in nude rats after acute myocardial infarction. Intravenous administration of *ex vivo* expanded human EPCs inhibited fibrosis and preserved function, and, as in the hindlimb ischemia model, the infused EPCs were recruited to the ischemic area and contributed to neovascularization (Kawamoto et al. 2001). Freshly isolated (i.e., not cultured) CD34+ EPCs produced similar results in rats with acute myocardial infarction (Kocher et al. 2001), and Taguchi et al. (2004) demonstrated that systemic infusion of human cord blood CD34+ EPCs augmented neovascularization in nude mice after cerebral infarction, thereby enhancing nerve regeneration and contributing to cerebral tissue repair.

The scarcity of EPCs in bone marrow and peripheral blood could limit the utility of EPC infusion in a clinical setting. To increase the effective dose of EPCs, our group administered EPC therapy via local transplantation rather than systemic infusion. In nude rats with acute myocardial ischemia, intramyocardial administration of human circulating CD34+ cells enhanced neocapillary formation, the reduction of infarct size, and functional preservation at a dose equivalent to 5% to 10% of the dose used for systemic infusion (Kawamoto et al. 2003). To simulate the clinical application of EPC transplantation, NOGA electromechanical mapping was used to guide intramyocardial transplantation of autologous EPCs in a swine model of chronic myocardial ischemia; the treatment was associated with significant attenuation of myocardial ischemia as well as functional improvement (Kawamoto et al. 2003).

Bone marrow or peripheral blood MNC preparations that contain a very low concentration (0.2–2%) of EPCs have been reported to enhance ischemic angiogenesis in both preclinical and clinical studies (Tateishi-Yuyama et al. 2002, Tateo et al. 2006). Mononuclear cells other than EPCs (e.g., CD34– cells) can contribute to ischemic neovascularization by secreting angiogenic cytokines (Kamihata et al. 2001); however, the MNCs from bone marrow comprise cells of mesenchymal as well as hematopoietic and endothelial lineages, so administration of the total MNC population could lead to undesirable cellular differentiation and tissue damage. Yoon et al. (2004) reported unexpected and severe myocardial calcification after intramyocardial transplantation of filtered bone marrow cells in a rat model of myocardial infarction. Recently, our lab compared the safety and efficacy of G-CSF-mobilized human CD34+ cells and total MNCs in a nude rat model of acute myocardial infarction. Both treatment groups received the same number of CD34+ cells. Compared to rats treated with purified CD34+ cells, MNC-treated rats displayed a higher incidence of moderate to severe hemorrhagic myocardial infarction accompanied by abundant infiltration of human CD45+ inflammatory cells 3 days after cell transplantation. Four weeks after transplantation, histological capillary density, fibrosis area, and echocardiographic functional parameters were better preserved in the group treated with CD34+ cells than in the MNC-treated group, despite equal dosing of CD34+ cells (Kawamoto et al. 2006a). These results indicate that CD34+ cells possess superior

potency for the preservation myocardial integrity and function after myocardial infarction (Figure 2). Clinical evidence of the superiority of CD34+ cells for myocardial neovascularization has been reported by Hoffman, et al. (2005). The investigators used 3D PET imaging to track infused radiolabeled bone marrow cells that were either unselected or enriched for CD34+. After intracoronary transfer, 14% to 39% of the CD34+ enriched cells were localized to the infarcted myocardium, compared to less than 3% of the unselected bone marrow cells.

The contribution of CD34+ cells to cardiovascular regeneration may not be limited to vascularization. Immunohistochemical and molecular analyses in our laboratory found evidence of CD34+ cell heritage among the cardiomyocytes and smooth muscle cells in the infarcted myocardium of rats (Iwasaki et al. 2006). These findings indicate that the preservation of cardiac function and structural integrity associated with EPC therapy includes both vasculogenic and myogenic components, although the relative contribution of each component has yet to be quantified.

Clinical Application of EPCs for Ischemic Neovascularization

The promising outcomes from preclinical studies of EPC transplantation lead to the initiation of several clinical trials (Table 1). In most trials, EPCs have been isolated from total MNCs via magnetic selection of CD34+ or AC133+ cells. After a pilot study demonstrated the safety and feasibility of the procedure (Stamm et al. 2003), Stamm et al. (2007) found that direct intramyocardial injection of autologous AC133+ cells during coronary artery bypass grafting surgery was associated with a favorable trend for improved cardiac perfusion and left ventricular function. The safety, feasibility, and efficacy of intramyocardial AC133+ cell transplantation has also been established for patients with chronic ischemic cardiomyopathy in the absence of bypass surgery (Klein et al. 2007). Our group performed a phase I/II, randomized, placebo-controlled, dose-ranging, clinical trial to evaluate the intramyocardial transplantation of G-CSF-mobilized CD34+ cells in 24 patients with intractable angina pectoris (Losordo et al. 2007). Patient-specific procedures included G-CSF injection, leukapheresis for cell harvesting, and NOGA mapping-guided cell injection, all of which were well tolerated with no severe adverse events reported. Favorable trends in angina frequency, exercise tolerance, and perfusion defect were observed in patients administered CD34+ cells compared with patients who received placebo. A phase IIb study is underway in the US.

The use of EPC therapy for treatment of acute or subacute myocardial infarction has been investigated by Assmus et al. (2002). Bone marrow total MNCs or an EPC-enriched population of cultured circulating progenitor cells were administered via intracoronary infusion to patients with acute myocardial infarction. Both treatments were associated with improved left ventricular function and regional wall motion. Despite a technical limitation in the culture method regarding EPC purity and the efficiency of expansion, these favorable results encourage future controlled, randomized, clinical trials. Bartunek et al. (2005) demonstrated the efficacy and feasibility of intracoronary AC133+ cell infusion in patients with recent myocardial infarction, although the therapy was associated with an increased incidence of coronary events.

Regarding the EPC application for patients with critical limb ischemia, our group treated 17 patients with intramuscular injection of autologous and G-CSF-mobilized CD34+ cells (Kawamoto et al. 2006b). The cell therapy was safely performed and the final report is now under preparation.

Future Perspectives

A decade has passed since the discovery of adult EPCs in human peripheral blood. During this period, several reports have demonstrated the potency of EPCs in preclinical studies of ischemia and myocardial infarction, and investigators are beginning to evaluate the therapeutic utility of EPCs in early-phase clinical trials. Many trials are likely to recruit patients with critical ischemia who have no other viable treatment options, and it may be difficult to establish significant efficacy in this fragile patient population. As an alternative approach, EPC therapy could be administered after successful primary stenting in patients with acute myocardial infarction; however, these “mild” cases of ischemia or heart failure may not display any notable improvement in prognosis or function with additional therapy. Thus, the success of future clinical investigations of EPC therapy is critically dependent on choosing an appropriate set of inclusion and exclusion criteria. Additional investigations designed to identify the appropriate cell type and dose and to optimize techniques for EPC isolation, expansion, mobilization, recruitment, and transplantation are also needed to continue the advancement of this novel therapeutic modality.

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Figure 1.

Mechanism of endothelial progenitor cell (EPC)-mediated postnatal neovascularization. Circulating EPCs mobilized from bone marrow are recruited into the foci of neovascularization and contribute to new blood vessel formation. The intrinsic EPC activity can be augmented by certain cytokines, growth factors, and pharmaceutical agents. G-CSF indicates granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; VEGF, vascular endothelial growth factor; SDF-1, stromal cell-derived factor-1.

Figure 2.

Representative histological assessments 3 days after intramyocardial injection of CD34+ cells or total mononuclear cells (MNCs). Experiments were performed in nude rats with acute myocardial infarction. Animals in both treatment groups were administered the same number of CD34+ cells. (a) Hematoxylin-eosin staining of the infarct area revealed massive hemorrhagic infarction (arrows) in rats administered total MNCs. (b) Immunostaining for human-specific CD45 to identify hematopoietic/inflammatory cells (arrows) in the infarcted myocardium. Human CD45+ cells were abundant in the total MNC treatment group, but not in the purified CD34+ cell treatment group.

Table 1
Clinical application of endothelial progenitor cells in coronary artery disease.

Study	Patients, n	Cell Type	Disease	Treatment	Control Group	Results
Stamm et al. (2003)	6	BM-AC133+	Chronic MI	IM with CABG	None	LVEF↑ Perfusion↑
Stamm et al. (2007)	40	BM-AC133+	Chronic MI	IM with CABG	CABG	LVEF↑ Perfusion↑
Klein et al.	10	BM-AC133+	Chronic ICM	IM	None	LVEF↑ NYHA class↓
Losordo et al.	24	GCSF-mobilized CD34+ cells	Chronic CAD	IM/NOGA	Placebo	Perfusion↑ CCS class↓
Assmus et al.	20	Cultured CPCs or BM-MNCs	Acute MI	IC	None	LVEF↑ Viability↑
Bartunek et al.	35	BM-AC133+	Recent MI	IC	Standard therapy	LVEF↑ Perfusion↑

BM indicates bone marrow; MI, myocardial infarction; IM, intramuscular injection; CABG, coronary artery bypass graft surgery; LVEF, left ventricular ejection fraction; ICM, ischemic cardiomyopathy; NYHA, New York Heart Association; GCSF, granulocyte colony stimulating factor; CAD, coronary artery disease; CCS, Canadian Cardiovascular Society; CPCs, circulating progenitor cells; IC, intracoronary injection; MNCs, mononuclear cells.