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Pro-angiogenic cell-based therapy for the treatment of ischemic cardiovascular diseases ☆

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A B S T R A C T

Pro-angiogenic cell therapy has emerged as a promising option to treat patients with acute myocardial infarction or with critical limb ischemia. Exciting pre-clinical studies have prompted the initiation of numerous clinical trials based on administration of stem/progenitor cells with pro-angiogenic potential. Most of the clinical studies performed so far have used bone marrow-derived or peripheral blood-derived mononuclear cells and showed, overall, a modest but significant benefit on tissue remodeling and function in patients with ischemic diseases. These mixed results pave the way for the development of strategies to overcome the limitation of autologous cell therapy and to propose more efficient approaches. Such strategies include pretreatment of cells with activators to augment cell recruitment and survival in the ischemic target area and/or the improvement of cell functions such as their paracrine ability to release proangiogenic factors and vasoactive molecules. In addition, efforts should be directed towards stimulation of both angiogenesis and vessel maturation, the development of a composite product consisting of stem/progenitor cells encapsulated in a biomaterial and the use of additional sources of regenerative cells.

Introduction

Insufficient organ perfusion following thrombotic vessel obstruction of the feeding artery is a major determinant of post-ischemic remodeling, ultimately leading to atrophy of the affected territory, important loss of function and serious health consequences. Although the prompt re-establishment of a patent artery has significantly reduced subsequent complications and mortality, deleterious remodeling still occurs since this therapy cannot be offered to a substantial proportion of patients with acute disease. In addition, insufficient neovascularization leading to tissue hypoperfusion is an integral component of tissue remodeling and loss of organ function following ischemic injury. Hence, therapeutic angiogenesis is viewed as a highly promising strategy to ensure revascularization of ischemic tissues by promoting the growth of new vessels or the maturation of pre-existing ones.

Advances in the field of vascular biology lead to the discovery of putative circulating endothelial progenitor cells (EPCs) in adults [1] and has triggered a massive amount of research regarding EPCs biology and their therapeutic potential for ischemic diseases, mainly in patients with acute myocardial (MI) or with critical limb ischemia (CLI), in the beginning of the past decade [2,3]. EPCs mainly originate from the bone marrow, but extramedullary EPCs can also be recruited towards ischemic tissues [4]. Consequently, whole bone marrow derived mononuclear cells (BMCs) or medullar cell selected on different markers (CD34+, CXCR4+, Lin-ckit+…) have often been used as source of EPCs for pre-clinical studies of cell therapy for therapeutic angiogenesis. Nevertheless, the true identity of EPCs is still under debate. Indeed, although efforts have recently been made to standardize the cell surface markers, isolation procedure and phenotypic properties that define bona-fide EPCs [5,2], a large number of different EPCs or EPCs-like populations have been used in experimental or clinical studies, and hamper a comprehensive understanding of the existing literature. Typically, these cells are defined on the basis of expression of cell surface markers such as CD34, Flk-1 and CD-133 but EPCs appear to be a heterogeneous group of cells originating from multiple precursors and present in different stages of endothelial differentiation in peripheral blood. At least two types of EPCs with divergent properties can be obtained in vitro [5]. "Early" EPCs possess a strong paracrine activity but no paracrine potential, while "Late" EPCs have low paracrine activity, but can incorporate into newly formed vessels [5]. Interestingly, both cell types can promote post-ischemic angiogenesis, and act synergistically when co-transplanted [6].

Mechanisms of EPCs-induced vascular regeneration

Although a substantial number of studies have demonstrated the pro-angiogenic and therapeutic effect of EPCs in experimental models of MI and CLI, the mechanisms of EPCs-induced neovascularization remain undefined [3]. After the seminal discovery of Asahara, the first mechanism of EPCs-induced angiogenesis to be proposed has been incorporation of EPCs into newly formed vascular structure, a process

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referred to as post-natal vasculogenesis [1]. However, a first critical point is the identification of cellular mechanisms governing progenitor cells ability to differentiate into EPC and subsequently, endothelial cells. Among these mechanisms, apoptotic or activated cells shed submicron microparticles (MPs) released after ischemia have been considered as endogenous signals leading to postischemic vasculogenesis. MPs from mice ischemic hind-limb muscle are detected by electron microscopy 48 hours after unilateral femoral artery ligation as vesicles of 0.1- to 1-microm diameter. After isolation by sequential centrifugation, flow cytometry analyses show that the annexin V(+) MPs concentration is 3.5-fold higher in ischemic calves than control muscles (1392+/−406 versus 394+/−180 annexin V(+) MPs per 1 mg; $P<0.001$) and come mainly from endothelial cells (71% of MPs are CD(144+)). MPs isolated from ischemic muscles induce more potent in vitro BMCs differentiation into cells with endothelial phenotype than those isolated from control muscles. MPs effects on postischemic revascularization were then examined in an ischemic hind-limb model. MPs isolated from ischemic muscles were injected into ischemic legs in parallel with venous injection of BMCs. MPs increase the proangiogenic effect of BMCs transplantation, and this effect is blunted by NOX2 deficiency. In parallel, BMCs proangiogenic potential also is reduced in ABCA1 knockout mice with impaired vesiculation. Hence, MPs produced during tissue ischemia stimulate progenitor cell differentiation and subsequently promote postnatal neovascularization [7].

A second critical point is that extensive studies have shown that EPCs incorporation into neovessels was generally low, and it appears nowadays that EPCs mainly act through the paracrine release of multiple factors and post-natal vasculogenesis cannot solely account for their pro-angiogenic effects [3]. Nevertheless, differentiation of bone marrow progenitors into cells of the vascular lineage is required for their long-term beneficial effect after myocardial infarction, as elimination of bone marrow progenitors expressing an inducible suicide gene under the control of an endothelial or smooth muscle cells specific gene promoter late after transplantation reverses the original therapeutic benefit [8]. The pro-angiogenic effect of EPCs might at least partially depend on paracrine signals, as EPCs express various pro-angiogenic cytokines such as VEGF or Interleukin-8 [5], and injection of a culture medium conditioned by EPCs recapitulates the pro-angiogenic and therapeutic effects of cell transplantation [9]. In addition, proangiogenic cells may also release vasoactive signals. BMCs and human EPCs derived from cord blood interact with ischemic femoral arteries through CXCL12 and CXCR4 signaling and release nitric oxide (NO) via an endothelial nitric oxide synthase (eNOS)-dependent pathway. BMCs and EPCs-induced NO production promote a marked vasodilation and disrupted vascular endothelial-cadherin/beta-catenin complexes, leading to increased vascular permeability. Of note, NO-dependent vasodilation and hyperpermeability are critical for progenitor cells infiltration in ischemic tissues and their proangiogenic potential in a model of hindlimb ischemia in mice [10]. In addition, EPCs also release proteases, such as cathepsin L, and promote a concomitant increase in matrix degradation that enables endothelial cell migration and vascular remodelling [11]. Intramyocardial delivery of BMCs in infarcted mice has been shown to regulate the expression of cardiac MicroRNAs (miRNAs) and down-regulate the proapoptotic miR-34a. Insulin Growth Factor-1 (IGF-1) significantly inhibits H(2)O(2)-induced miR-34a expression, and miR-34a overexpression abolishes the antiapoptotic effect of IGF-1 suggesting that BMCs release IGF-1, which inhibits the processing of miR-34a, thereby blocking cardiomyocyte apoptosis [12]. Finally, EPCs can also promote tissue repair and post-MI angiogenesis by inducing recruitment of endogenous bone marrow derived cells or progenitor cells localized in the ischemic tissue [13–15].

Clinical trials

After the discovery of EPCs and a tremendous amount of experimental evidence pointing towards EPCs or BMCs therapeutic potential for the treatment of MI [16], clinical trials evaluating the benefit of intracoronary administration of BMCs for therapeutic angiogenesis have been conducted in patients with ischemic diseases [17,18,18–23]. However, clinical trials showed mixed results. For example, although Schachinger et al demonstrated that intracoronary BMCs delivery early after MI (3 to 7 days) improved cardiac function at 4 months and reduced death, recurrence of MI and need for a revascularization procedure at 1 year [18], a simultaneously published trial did not show any benefit of early BMCs transplantation at 6 months of follow-up [19]. Moreover, the BOOST trial evidenced a benefit of BMCs transplantation after 6 months that was lost at 18 months follow up [24]. Recently, intracoronary transplantation of BMCs 2 to 3 weeks after MI do not produce any functional benefits [25]. Nevertheless, a 2008 meta-analysis of 13 trials with a total of more than 800 patients has shown that BMCs transplantation led to a modest (+2.99%) but significant increase in Left Ventricle (LV) ejection fraction in patients receiving BMCs when compared to placebo [26]. In this line, recently, a total of 50 studies (enrolling 2,625 patients) identified by database searches through January 2012 were analyzed. Transplantation of adult BMCs improves LV function, infarct size, and remodeling in patients with ischemic heart disease compared with standard therapy, and these benefits persist during long-term follow-up. BMCs transplantation also reduces the incidence of death, recurrent MI, and stent thrombosis in patients with ischemic heart disease.

Activation of adult stem/progenitor cells regenerative potential

The use of autologous cells is fraught with several hurdles, particularly their often defective functionality in patients with atherosclerosis diseases. Indeed, more than being just causes for coronary artery disease and the onset of MI or CLI, cardiovascular risk factors such as hypertension [27], diabetes [28,29] or hyperlipidemia profoundly impair endogenous and therapeutically induced post-ischemic angiogenesis, and represent a major difficulty to circumvent in pro-angiogenic therapy [30]. Along this line, several reports have highlighted bone marrow progenitor cell deficiency in diabetic [31], dyslipidemic [32] patients or in hypertension [33,34]. Hence, in a model of hindlimb ischemia, basal postischemic neovascularization is reduced in Spontaneously Hypertensive Rats (SHR) compared to normotensive animals (WKY). Treatments with Angiotensin Converting Enzyme (ACE) inhibitor (perindopril) or angiotensin type 1 receptor blocker (Losartan) or cotreatment with ACE inhibitor and diuretic (indapamide) decrease blood pressure levels and restore vessel growth in SHR to WKY levels. Interestingly, 14 days after BMCs transfusion, angiographic scores, capillary density, and foot perfusion are decreased by 1.4-, 1.5-, and 1.2-fold, respectively in SHR transfused with BMCs isolated from SHR compared to those receiving BMCs of WKY. Alteration in BMCs proangiogenic potential is likely related to the reduction in their ability to mobilize into peripheral circulation, as revealed by the 2.9-fold decrease in number of circulating CD34+/CD117+ cells and to differentiate into cells with endothelial phenotype, as revealed by the 2.1-fold reduction in percentages of DiILDL/BS-1 lectin positive cells. In addition, reactive oxygen species (ROS) levels are increased by 2.2-fold in SHR BMCs compared to WKY BMCs, as assessed by L-012 luminescence. Cotreatment with ACE inhibitor, angiotensin type 1 receptor blocker, or ACE inhibitor and diuretic or antioxidants (NAC 3 mmol/L, Apocynin 200 micromol/L) reduced ROS levels, improved the number of DiILDL/BS-1 lectin-positive cells and restored BMCs proangiogenic effects in ischemic hindlimb [33,34].

The alternate use of banked allogeneic cells would provide a more consistent and readily available product but the expectedly rapid rejection of these cells could only be avoided by an immunosuppressive treatment which carries its own safety risks. Therefore, our effort should be moved towards the development of strategies that may circumvent stem/progenitor cells dysfunction.