Current status of cell therapy for systemic arterial hypertension

Expert Rev. Cardiovasc. Ther. 7(11), 1307–1311 (2009)

Valdo Jose Dias Silva
Department of Biological Sciences, School of Medicine, Triangulo Mineiro Federal University, Uberaba, MG, 38015-050, Brazil
Tel.: +55 343 318 5421
Fax: +55 343 318 5466
valdo@mednet.com.br

Marcus Paulo Ribeiro Machado
Department of Biological Sciences, School of Medicine, Triangulo Mineiro Federal University, Uberaba, MG, 38015-050, Brazil
Tel.: +55 343 318 5421
Fax: +55 343 318 5466
mprmachado@yahoo.com.br

Julio Cesar Voltarelli
Author for correspondence
Department of Clinical Medicine, School of Medicine of Ribeirao Preto, University of Sao Paulo, Ribeirao Preto, SP, 14049-900, Brazil
Tel.: +55 162 101 9369
Fax: +55 162 101 9372
jcvoltar@fmrp.usp.br

"Systemic arterial hypertension affects approximately a fifth of the world's total population … at least 65 million adult Americans (nearly a third of the US adult population) have hypertension."

Systemic arterial hypertension (SAH) affects approximately a fifth of the world's total population and is a huge public health problem. According to the National Health and Nutrition Examination Survey (NHANES), at least 65 million adult Americans (nearly a third of the US adult population) have hypertension, defined as a systolic blood pressure greater than 140 mmHg and diastolic blood pressure greater than 90 mmHg, and/or current use of antihypertensive medication [1]. The worldwide prevalence of hypertension, which rises progressively with age, may be as many as 1 billion individuals, causing approximately 7.1 million deaths per year [1].

Although several advances have been made in understanding the pathophysiological basis of essential arterial hypertension, which represents 90–95% of cases of SAH, the complete framework of its pathogenesis is not completely understood. An enormous and efficacious therapeutic arsenal is currently available to treat arterial hypertension and its consequences on target organs [2], but the disease does not yet have a definitive cure, making it imperative that we search for new therapeutic modalities.

Regarding alternative and more effective treatments for SAH, gene therapy has been investigated intensively, mainly in an experimental context using animal models [3]. Nevertheless, problems regarding safety and efficacy need to be surmounted in order to consider a clinical use of this new therapy.

The mechanisms associated with a rise in arterial blood pressure are mainly related to a marked increase in total peripheral resistance, accompanied by a normal or even reduced cardiac output [4]. One of the most important causes of the increased total peripheral resistance in arterial hypertension is the marked sympathetic overactivity, which has been considered a hallmark of essential hypertension in humans as well as in an experimental animal model of spontaneous genetic hypertension, the spontaneously hypertensive rat (SHR) [5]. Besides sympathetic overactivity, several other changes have been described in SAH, such as tissue renin–angiotensin system hyperactivity, altered renal sodium handling, and endothelial dysfunction [6–8].

Accentuated endothelial dysfunction has been described in arterial hypertension in both humans and experimental animals [7,8] and is directly implicated in the pathogenesis of increased total peripheral resistance and also in chronic lesions of target organs [7,8]. Another marked characteristic of SHR and humans with essential hypertension is the microvascular rarefaction, which is at least partially due to, or is associated with, the endothelial dysfunction [9]. This microvascular rarefaction results in significant loss of arteriolar and capillary vessels arranged in a parallel way in the microcirculation, which certainly contributes to the elevated total peripheral resistance observed in hypertensive states [9]. The causes of this microvascular rarefaction are not completely elucidated, but seem to involve defective angiogenesis [9,10].

Angiogenesis, the formation of new microvessels from pre-existing endothelial cells, is a complex phenomenon mediated by

EXPERT REVIEWS

 10.1586/ERC.09.110 ISSN 1477-9072

several signaling molecules and growth factors, such as VEGF, and angiopoietin 1 and 2, among others [10]. Wang *et al.* have recently demonstrated in SHR a reduced expression of VEGF receptor 2 (VEGFR-2), also known as kinase insert domain receptor (KDR), the most important receptor mediating the angiogenic signaling of VEGF, in association with a reduced expression of membrane type-1 matrix metalloproteinase (MT1-MMP), implicated in the tissue invasion ability of endothelial cells during angiogenesis [11].

In recent years, it has become clear that the formation of new microvessels is not only dependent on angiogenesis, but also includes the recruitment of bone marrow-derived endothelial progenitor cells (EPCs), a phenomenon termed postnatal vasculogenesis [12]. These circulating cells can augment the formation of capillaries by homing and incorporation into the endothelium of the growing vessels in a process that represents a postnatal form of vasculogenesis [12]. They also show the expression of various endothelial markers, and incorporate into neovessels at sites of ischemia [12]. Asahara's and Rafii's groups in 1997 and 1998 [13,14] reported that the circulating bone marrow-derived EPCs in the adult were a subset of $CD34^+$ hematopoietic stem cells, also expressing an endothelial marker protein, VEGFR2. Since CD34 is not exclusively expressed on hematopoietic stem cells but, albeit at a lower level, also on mature endothelial cells, further studies used the more immature hematopoietic stem cell marker CD133 to characterize this cellular compartment [15]. Thus, $CD133^+VEGFR2^+$ cells more likely reflect immature progenitor cells, whereas $CD34^+VEGFR2^+$ may also represent shedded cells from the vessel wall. In the last few years, the nature and role of EPCs in vascular maintenance and repair have been investigated extensively [12]. Circulating EPCs are reduced in number and function in patients at risk for cardiovascular disease [12]. EPC dysfunction is strongly associated with endothelial dysfunction and a possible predictive marker for future cardiovascular events [12]. Since essential arterial hypertension is also associated with endothelial dysfunction, we may speculate that impaired EPC function could contribute to the development of the microvascular abnormalities that lead to increased vascular resistance in SAH.

> "...the formation of new microvessels is not only dependent on angiogenesis, but also includes the recruitment of bone marrow-derived endothelial progenitor cells, a phenomenon termed postnatal vasculogenesis."

The first evidence for EPC dysfunction in SAH was reported by Vasa *et al.*, in 2001, showing an impairment of migratory capacity of EPCs despite nonaltered number of these cells in the peripheral blood of a hypertensive patient subset [16]. In 2005, Imanishi *et al.* demonstrated an accelerated senescence of circulating EPCs in both experimental hypertensive rats and patients with essential hypertension [17]. Werner *et al.* have also found a significant correlation between levels of $CD34^+/KDR^+$ (VEGF2) EPCs and the occurrence of cardiovascular events and death from cardiovascular causes [18]. Surprisingly, in a subgroup of 432 coronary artery disease patients with arterial hypertension, no association was observed between the number of $CD34^+/KDR^+$ cells and arterial hypertension, suggesting that the EPC dysfunction may actually be of greater importance than the number of circulating EPCs in patients with hypertension [18]. However, a reduced number of circulating EPCs in hypertensive patients has been found in recent reports [19–21], suggesting that reduced number or dysfunction of EPCs may play a significant role in the pathogenesis of endothelial dysfunction, microvascular rarefaction and target-organ damage associated with SAH.

> "...endothelial progenitor cells dysfunction may actually be of greater importance than the number of circulating endothelial progenitor cells in patients with hypertension."

The reasons for the impairment of EPC number or function in arterial hypertension have not yet been completely understood, but recent reports seem to implicate an augmented oxidative stress associated with renin–angiotensin system overactivity, causing accelerated senescence or apoptosis of EPCs [22]. In addition, defective VEGF–KDR signaling pathways observed in experimental hypertensive rats [11] could be involved in the EPC dysfunction associated with SAH. Several experimental and clinical studies have suggested that angiotensin-converting enzyme (ACE)-inhibitors, angiotensin type 1 (AT-1) receptor antagonists, statins, PPAR-γ agonists, erythropoietin and VEGF increase the number and functional activity of EPCs [12]. The underlying mechanisms remain largely to be defined; however, they likely include inhibition of NAD(P)H oxidase activity of progenitor cells after renin–angiotensin system inhibition, as well as activation of the PI3-kinase/Akt pathway and endothelial nitric oxide synthase [12].

In recent years, new therapeutic modalities for cardiovascular diseases have emerged based on cell transplantation (cell therapy) [23]. Regarding cell therapy for cardiovascular diseases, significant achievements have been made using stem cells with pluri- or multi-potential properties to treat several cardiovascular conditions in experimental and also in the clinical context [23].

Taking into consideration that endothelial dysfunction and microvascular rarefaction are marked alterations in the pathogenesis of SAH [7–10], it could be hypothesized that the reduced number and/or function of EPCs in arterial hypertension could benefit from reposition (transplantation) and/or mobilization of EPCs, which could induce some reduction in arterial blood pressure levels and improve endothelial dysfunction and microvascular rarefaction.

In the literature, no report was found relating to the autologous transplantation of EPCs or other types of adult stem cells for treatment of high blood pressure levels, endothelial dysfunction or microvascular rarefaction in SAH. In fact, only one study was found in the literature in which stem cells were used with this specific purpose [24], showing a chronic antihypertensive effect (30 days) after only one administration of allogenic embryonic stem cells in SHRs [24]. However, no mechanistic explanation for this antihypertensive effect was investigated in this report.

Nagaya *et al.* have demonstrated that only one injection of human EPCs, transplanted to immunodeficient nude rats with monocrotaline-induced pulmonary hypertension, was able to reduce pulmonary hypertension and to improve survival of the animals [25]. Similar findings were reported by other researchers using different adult stem cells transduced or not with different genes to treat pulmonary hypertension [26]. All these observations give support to the hypothesis that the same phenomenon could happen in SAH, despite marked differences in the pathophysiology of pulmonary and systemic forms of arterial hypertension.

> "...only one injection of human endothelial progenitor cells, transplanted to immunodeficient nude rats with monocrotaline-induced pulmonary hypertension, was able to reduce pulmonary hypertension and to improve survival of the animals."

Additional support for this hypothesis was lent by the recent report by Wassmann *et al.* [27] in which intravenous transfusion of 2×10^7 spleen-derived mononuclear cells (MNCs) isolated from wild-type mice restored endothelium-dependent vasodilation in apoE$^{-/-}$ hypercholesterolemic mice. We may hypothesize that a similar behavior could be found after transfusion of bone marrow mononuclear or progenitor/stem cells for hypertensive animals or humans, since endothelial dysfunction is also a hallmark of SAH.

Several lines of evidences have demonstrated that bone marrow stem cells such as EPCs and mesenchymal stem cells are able to release several growth factors and cytokines, which, by means of a paracrine action, could be acting on the neighboring cells stimulating proliferation, survival and angiogenesis while inhibiting apoptosis, fibrosis and oxidative stress [28]. Among the growth factors already described, as secreted by bone marrow cells, we may find VEGF, HGF, IGF-1 and adrenomedullin. Almost all of these factors presented antihypertensive actions when injected intravenously [28], reinforcing the idea that bone marrow progenitor/stem cells could be effective in reducing systemic arterial pressure by means of a paracrine effect on vessels.

Some reports in the literature have described the great resistance of the progenitor/stem cells to oxidative stress, since they express large quantities of intracellular antioxidants or free radical scavengers such as, superoxide dismutase, thioredoxin, catalase and glutathione oxyreductase [29]. Here we may identify another potential antihypertensive effect of bone marrow cells; reducing the increased vascular oxidative stress that is another hallmark of the hypertensive state.

Even though no follow-up of high blood pressure levels or endothelial dysfunction has been undertaken, two recent papers have demonstrated the beneficial effects of bone marrow EPC cells in experimental hypertensive animals, improving the neovascularization capacity in a model of ischemic hindlimbs in SHR [30] and ameliorating renal hemodynamics and function *in vivo* in pigs with renal artery stenosis [31]. These studies support the use of EPCs as a therapeutic intervention for peripheral artery disease in hypertensive patients or renovascular hypertensive disease.

Regarding mesenchymal stem cells, another type of bone marrow stem cells, two recent reports from Nardi's group have described beneficial effects, topically or systemically administered, in spontaneously hypertensive rats with congestive heart failure due to myocardial infarction after coronary artery ligature [32,33]. A reduction of myocardial remodeling associated with an improvement in echocardiographic parameters was observed after 4 weeks of treatment with bone marrow-derived mesenchymal stem cells. However, these authors did not follow-up the high blood pressure levels or endothelial dysfunction in control or infarcted SHRs.

Despite this potential evidence, Perry *et al.* recently reported that transplantation of eGFP$^+$-labeled bone marrow cells in endothelial nitric oxide synthase-deficient (eNOS$^{-/-}$) mice with previous radiation- or busulfan-induced bone marrow ablation was not able to repair endothelium dysfunction or reduce arterial blood pressure in this mouse model of chronic endothelial dysfunction and arterial hypertension [34]. These results argue against previous reports that indicate a physiological role played by circulating bone marrow-derived EPCs on endothelium homeostasis. However, the lack of renewal of chronic dysfunctional endothelium observed by in eNOS$^{-/-}$ mice in this study could be due to an impairment of primitive EPC homing in bone marrow after transplantation, since eNOS-derived nitric oxide seems to be essential to the homing of bone marrow cells into their niches [35]. Bone marrow transplantation in mieloablated hypertensive animals with some degree, not absence of, eNOS expression should be addressed in order to better clarify this issue.

> "A reduction of myocardial remodeling associated with an improvement in echocardiographic parameters was observed after 4 weeks of treatment with bone marrow-derived mesenchymal stem cells."

Considering all of these previous reports and the clinical and epidemiological relevance of SAH, the utilization of bone marrow mononuclear or progenitor/stem cells to treat SAH seems to be compellingly attractive. In order to test this hypothesis, we have performed a series of experiments in which bone marrow mononuclear cells [36] or a subpopulation of these cells, expressing the markers CD133 and KDR (VEGFR2), which phenotypically mark the EPCs, were intravenously administrated to 16-week-old SHRs with ongoing hypertension [Dias da Silva, Unpublished Data]. Unfractionated bone marrow mononuclear cells, as well as CD133$^+$/KDR$^+$ cells, were able to reduce the arterial blood pressure by at least 20 mmHg over at least 15 days, which was associated with an improvement in endothelial dysfunction, as measured by acethylcholine-induced endothelium-dependent vasodilation and also with lower cardiac hypertrophy, which could be due to a reduction in the hemodynamic overload. Fluorescence-labeled, exogenously

applied progenitor cells were found attached to the endothelial cell layer of capillary and arteriolar vessels in the kidney as well as in the spleen, and to a lesser extent in other organs, including the lungs, liver and heart, for example.

Although these preliminary data did not present any mechanistic explanation, to our knowledge they are the first demonstration of the antihypertensive effect of bone marrow cells or EPCs in an experimental model of SAH, suggesting that bone marrow cell transplantation could become, in the near future, a new potential therapeutic modality to treat SAH and endothelial dysfunction, contributing to a significant and prolonged reduction in tensional levels in hypertensive states.

Financial & competing interests disclosure

This article was supported by CNPq (National Council for Scientific and Technological Development), grant numbers 478698/2004-6, 573754/2008-0 and 573638/2008-9; FAPEMIG (Research Foundation of Minas Gerais State), grant numbers CBB1437/2005 CBB3332-3.13/2007; and FAPESP/CTC/CEPID grant number 573638/2008-9. Marcus Paulo Ribeiro Machado was supported by a doctoral fellowship from FAPEMIG, Brazil. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

1 Cooper RS, Tayo B. Geographic patterns of hypertension: a global perspective. In: *Hypertension Primer: The Essentials of High Blood Pressure.* 4th Edition. Izzo, JL, Sica DA, Black HR (Eds). Lippincott, Williams and Wilkins, PA, USA, 239–241 (2008).

2 Chobanian AV, Bakris GL, Black HR *et al.* Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 42(6), 1206–1252 (2003).

3 Pravenec M, Kurtz TW. Molecular genetics of experimental hypertension and the metabolic syndrome: from gene pathways to new therapies. *Hypertension* 49(5), 941–952 (2007).

4 Coleman TG, Hall JE. Systemic hemodynamics and regional blood flow regulation. In: *Hypertension Primer: The Essentials of High Blood Pressure.* 4th Edition. Izzo, JL, Sica DA, Black HR (Eds). Lippincott, Williams and Wilkins, PA, USA, 129–132 (2008).

5 Judy WV. Watanabe AM. Henry DP *et al.* Sympathetic nerve activity: role in regulation of blood pressure in the spontaneously hypertensive rat. *Circ. Res.* 38(Suppl. 2), 21–29 (1976).

6 Brooks VL, Haywood JR, Johnson AK. Translation of salt retention to central activation of the sympathetic nervous system in hypertension. *Clin. Exp. Pharmacol. Physiol.* 32(5–6), 426–432 (2005).

7 McIntyre M, Bohr DF, Dominiczak AF. Endothelial function in hypertension: the role of superoxide anion. *Hypertension* 34, 539–545 (1999).

8 Touyz RM. Reactive oxygen species, vascular oxidative stress, and redox signaling in hypertension: what is the clinical significance? *Hypertension* 44, 248–252 (2004).

9 Levy BI, Ambrosio G, Pries AR *et al.* Microcirculation in hypertension: a new target for treatment? *Circulation* 104, 735–740 (2001).

10 le Noble FA, Stassen FR, Hacking WJ *et al.* Angiogenesis and hypertension. *J. Hypertens.* 16, 1563–1572 (1998).

11 Wang H, Olszewski, B, Rosebury W *et al.* Impaired angiogenesis in SHR is associated with decreased KDR and MT1-MMP expression. *Biochem. Biophys. Res. Comm.* 315, 363–368 (2004).

12 Murasawa S, Asahara T. Endothelial progenitor cells for vasculogenesis. *Physiology (Bethesda)* 20, 36–42 (2005).

13 Asahara T, Murohara T, Sullivan A *et al.* Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275(5302), 964–967 (1997).

14 Shi Q, Rafii S, Wu MH *et al.* Evidence for circulating bone marrow-derived endothelial cells. *Blood* 92(2), 362–367 (1998).

15 Peichev M, Naiyer AJ, Pereira D *et al.* Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 95, 952–958 (2000).

16 Vasa M, Fichtlscherer S, Aicher A *et al.* Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ. Res.* 89, E1–E7, 2001.

17 Imanishi T, Moriwaki C, Hano T *et al.* Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. *J. Hypertens.* 23(10), 1831–1837 (2005).

18 Werner N, Kosiol S, Schiegl T *et al.* Circulating endothelial progenitor cells and cardiovascular outcomes. *N. Engl. J. Med.* 353(10), 999–1007 (2005).

19 Pirro M, Schillaci G, Menecali C *et al.* Reduced number of circulating endothelial progenitors and HOXA9 expression in CD34+ cells of hypertensive patients. *J. Hypertens.* 25(10), 2093–2099 (2007).

20 Oliveras A, Soler MJ, Martínez-Estrada OM *et al.* Endothelial progenitor cells are reduced in refractory hypertension. *J. Hum. Hypertens.* 22(3), 183–190 (2008).

21 Umemura T, Soga J, Hidaka T *et al.* Aging and hypertension are independent risk factors for reduced number of circulating endothelial progenitor cells. *Am. J. Hypertens.* 21(11), 1203–1209 (2008).

22 Imanishi T, Hano T, Nishio I. Angiotensin II accelerates endothelial progenitor cell senescence through induction of oxidative stress. *J. Hypertens.* 23(1), 97–104 (2005).

23 Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: the scientific foundations of cardiac repair. *J. Clin. Invest.* 115(3), 572–583 (2005).

24 Kukharchuk OL, Radchenko VV, Sipman BM *et al.* Effect of allotransplantation of embryonic pluripotent progenitor cells on dynamics of systemic arterial blood pressure in spontaneously hypertensive rats. *Fiziol. Zh.* 49, 68–71 (2003).

25 Nagaya N, Kangawa K, Kanda M *et al.* Hybrid cell-gene therapy for pulmonary hypertension based on phagocytosing action of endothelial progenitor cells. *Circulation* 108, 889–895 (2003).

26 Marsboom G, Janssens S. Endothelial progenitor cells: new perspectives and applications in cardiovascular therapies. *Expert Rev. Cardiovasc. Ther.* 6(5), 687–701 (2008).

27 Wassmann S, Werner N, Czech T *et al.* Improvement of endothelial function by systemic transfusion of vascular progenitor cells. *Circ. Res.* 99(8), e74–e83 (2006).

28 Gnecchi M, Zhang Z, Ni A, Dzau VJ. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ. Res.* 103(11), 1204–1219 (2008).

29 Watson T, Goon PK, Lip GY. Endothelial progenitor cells, endothelial dysfunction, inflammation, and oxidative stress in hypertension. *Antioxid. Redox Signal.* 10(6), 1079–1088 (2008).

30 de Nigris F, Balestrieri ML, Williams-Ignarro S *et al.* Therapeutic effects of autologous bone marrow cells and metabolic intervention in the ischemic hindlimb of spontaneously hypertensive rats involve reduced cell senescence and CXCR4/Akt/eNOS pathways. *J. Cardiovasc. Pharmacol.* 50(4), 424–433 (2007).

31 Chade AR, Zhu X, Lavi R *et al.* Endothelial progenitor cells restore renal function in chronic experimental renovascular disease. *Circulation* 119(4), 547–557 (2009).

32 de Macedo Braga LM, Lacchini S, Schaan BD *et al.* In situ delivery of bone marrow cells and mesenchymal stem cells improves cardiovascular function in hypertensive rats submitted to myocardial infarction. *J. Biomed. Sci.* 15(3), 365–374 (2008).

33 Braga LM, Rosa K, Rodrigues B *et al.* Systemic delivery of adult stem cells improves cardiac function in spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.* 35(2), 113–119 (2008).

34 Perry TE, Song M, Despres DJ *et al.* Bone marrow-derived cells do not repair endothelium in a mouse model of chronic endothelial cell dysfunction. *Cardiovasc. Res.* DOI: 10.1093/cvr/cvp215 (2009) (Epub ahead of print).

35 Aicher A, Heeschen C, Mildner-Rihm C *et al.* Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat. Med.* 9(11), 1370–1376 (2003).

36 Dias da Silva VJ, Machado MP, Rocha AM *et al.* Bone marrow stem/progenitor cells reduce arterial blood pressure and ameliorate endothelial dysfunction in spontaneously hypertensive rats. *FASEB J.* (Abstract) 19, 7439–7439 (2005).