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Bone marrow stem cells and their role in angiogenesis

Células-tronco da medula óssea e seu papel na angiogênese

Paulo Eduardo Ocke Reis*

Abstract

The degree of symptomatology of a patient with peripheral arterial disease dictates the kind of treatment. Despite the known therapies, some patients continue to have pain with ambulation, which affects their quality of life. The therapeutic implications of the angiogenic growth factors were identified by the pioneering studies of Folkman et al. 2 decades ago. Further investigations established the possibility of the use of formulations of recombinant angiogenic growth factors, with the objective of developing or increasing the network of collaterals in animal models of chronic myocardial or limb ischemia. Researches suggest that primitive stem cells with whole bone marrow possess greater functional plasticity, capable of contributing to regeneration of ischemic limb muscle and vascular endothelium by adult stem cells. Local autologous marrow stromal cells implantation induces a neovascular response resulting in a significant increase in blood flow to the ischemic limb. In this article we review the studies that have established how the implantation of bone marrow cells into ischemic limbs increases collateral vessel formation.

Key words: growth factor, angiogenesis factor, stem cells, ischemia.

Resumo

O nível de sintomatologia de um paciente com doença arterial periférica determina o tipo de tratamento. Apesar das terapias conhecidas, alguns pacientes continuam a ter dor com a deambulação, o que afeta sua qualidade de vida. As implicações terapêuticas dos fatores de crescimento angiogênicos foram identificadas por estudos pioneiros de Folkman et al. há 2 décadas. Investigações posteriores estabeleceram a possibilidade de utilizar formulações de fatores de crescimento angiogênico recombinantes, com o objetivo de desenvolver ou aumentar a rede de colaterais em modelos animais de isquemia crônica ou miocárdica. Pesquisas sugerem que células-tronco primitivas com medula óssea possuem maior funcionalidade plástica, capazes de contribuir com a regeneração de músculos isquêmicos e endotélio vascular por células-tronco adultas. O implante local e autólogo de células estromais da medula induz a uma resposta neovascular, resultando em um aumento significativo de fluxo sanguíneo no membro isquêmico. Neste artigo, revisamos os estudos que estabeleceram como o implante de células da medula óssea em membros isquêmicos aumenta a formação de vasos colaterais.

Palavras-chave: fator de crescimento, fator de angiogênese, células-tronco, isquemia.

The present treatment of critical ischemia of the lower limbs consists of a universal and aggressive attempt to save the ischemic limb. In spite of the knowledge of the factors that contribute to the development of lower limb ischemic disease, some limitations are found, mainly in the identification of the amputation risk population, and in the prevention of the advance of the vascular disease. The risk factors identified for peripheral arterial occlusive disease (PAOD) are essentially the same as for arteriosclerosis. Smoking patients present

three times more risk of developing ischemic symptoms in the lower limbs than non-smoking patients. Patients with diabetes have twice the risk of developing peripheral arterial disease, compared to non-diabetic patients. Resistance to insulin, glucose intolerance and hyperinsulinism were considered risk factors for PAOD. Also, the association of hypertriglyceridemia with the progression of carotid and coronary arteriosclerosis is well established.¹ The role of systemic arterial hypertension in the development of PAOD is debatable, with the Framingham and Finnish studies reaching opposite conclusions. It is known that the aggressive control of blood pressure in recently diagnosed hypertensive patients can reduce perfusion, causing decompensation of the limb, making it symptomatic. When the risk factors above coexist, the risk of developing PAOD substantially increases. It is important to stress the growing mortality in the population with peripheral

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vascular disease due to the influence of the ischemic limb on the cardiovascular system.

The main clinical manifestation of the disease is intermittent claudication. In a period of 5 to 10 years, about 70% of these patients will remain asymptomatic or oligosymptomatic, but 20 to 30% will show progressive symptoms and have indications of arterial revascularization. Some 15 to 20% will evolve from intermittent claudication to critical limb ischemia, and about 5 to 10% will have indication of amputation, with an incidence of less than 4% for major amputation.

Numerically, about 3,200 to 4,800 people/1,000,000 inhabitants/year will be subjected to a major amputation. The presence of diabetes mellitus represents the greatest risk, and the association of this with the use of tobacco provides a faster evolution. Dormandy et al. demonstrated that an ankle-arm index (ABI) of 0.5 in the initial diagnosis is the most significant prognostic indicator for the PAOD deterioration and the need for intervention.²

For the various steps in the progress of arterial disease there are different therapeutic behaviors. Oligosymptomatic or those with claudication that does not generate alterations in the everyday life should be treated conservatively through habit modification and the use of vasoactive medication.

Whereas patients with incapacitating claudication for short distances show a great restriction in their habits, patients showing pain when at rest or with ischemic lesions in the lower limbs are candidates for revascularization.

More recently, alternatives related to cellular therapy started to be developed. In the last 4 years, some *in vitro* and *in vivo* studies have been published, suggesting the potential of the use of stem cells (SC) for this purpose. The autologous transplantation of bone marrow mononuclear cells (ATBMMC) has shown great therapeutic potential *in vitro* as well as *in vivo*. Angiogenesis induced by the treatment with stem cells, coupled with the practice of exercises and pharmaceutical therapy can be a therapeutic alternative for patients with PAOD.

Clinical trials

The therapeutic implications of the angiogenic growth factors were identified by the pioneering studies of Folkman et al. 2 decades ago.³ Further investigations established the possibility of the use of formulations of

recombinant angiogenic growth factors, with the objective of developing or increasing the network of collaterals in animal models of chronic myocardial or limb ischemia. This new strategy was called angiogenesis. Takeshida et al.⁴ investigated the hypothesis that the vascular endothelial growth factor (phVEGF) was sufficient to constitute a therapeutic effect. His group used a single intraarterial bolus of VEGF and established proof of the angiogenic activity of VEGF in a rabbit ischemic hindlimb model.⁴ Preclinical findings suggest that intraarterial gene transfer of a plasmid can improve blood supply to the ischemic limb. The genic transfection in humans using DNA bearing phVEGF was initially carried out successfully for treating patients with severe limb ischemia.⁵ Three patients with pain at rest and treated with 1,000 µg phVEGF evolved with improved symptoms and arterial flux for the treated member, after a 1-year follow-up. With the dosage increased to 2,000 µg, angiographic and histologic evidence of vascular neoformation became evident. The interpretation is that the administration of endothelial cells mitogens promotes angiogenesis in patients with limb ischemia.⁵ Those investigators tested the hypothesis that endothelial nitric oxide synthase modulates angiogenesis in two rabbit ischemic hindlimb model, in which therapeutic angiogenesis has been showed as a compensatory response to tissue ischemia. Angiogenesis in the ischemic hindlimb was improved; there was angiographical evidence of vascularity in the limb.⁶

Precursor endothelial cells, originating in the bone marrow, could be identified as CD34+ and VEGFR2+, although other markers such as AC133+ and CD31+ have been described. During the tissue ischemia, with a drop in the oxygen levels, there is an increase in the production of HIF-1, which in its turn will unleash the increase of several growth factors, notably VEGF. Asahara et al. isolated putative endothelial cell progenitors from peripheral blood by magnetic bead selection on the basis of cell surface CD34+ antigen expression that became spindle-shaped endothelial cells and proliferated for 4 weeks.⁷ Bone marrow contains pluripotent CD34+ cells, which are known to give rise to hematopoietic cells. *In vitro* studies show that they can differentiate into mature endothelial cells.^{7,8} A study demonstrated in the dog that CD34+ cells seeded into grafts could enhance vascular graft endothelialization and vessel formation.⁹

Other alternatives have been developed based on the conceptual notion that endothelial cells and

hematopoietic stem cells come from a common precursor: the hemangioblast.¹⁰ Therefore, the paradigm that endothelial cells were generated by replication of mature endothelial cells was revolutionized. This author observed that a great part of the cells involved in the process of angiogenesis had their origin in the bone marrow (BM).¹⁰

The BM has endothelial progenitor cells that secrete several growth factors and can contribute to the formation of new capillaries. Authors investigated the extent of angiogenesis induced by implantation of autologous cells. In a rat ischemic hindlimb model, using nonradioactive colored microspheres and by determining the femoral arteriovenous oxygen difference postligation at 2 weeks, they showed the severity of the ischemic insult. To assess angiogenesis, histologic evaluation and angiography were done. They concluded that BM cells induced angiogenesis and improved deteriorated exercise capacity in the animal.¹¹ Also, the angiogenic effect was examined in the ischemic hindlimb in a diabetic rat model. Diabetes mellitus was induced by streptozotocin.¹²

Direct myocardial injection of phVEGF-A₁₆₅ was carried out successfully in five patients with coronary disease and no possibility of revascularization.¹³ Another study carried out on seven patients suffering from chronic refractory angina, by means of a minithoracotomy, through transepipcardiac injections, achieved the same objective. Other studies with placebo controls were published in the USA. In 2002 the second genic therapy in humans (AGENT), randomized, double blind and controlled by placebo, in patients with stable angina class Canadian II and III was published. The increase in VEGF will be the main stimulus for the mobilization of the BM cells as well as the main signal for homing these cells onto the ischemic tissues, and their later differentiation into endothelial cells in tubular structures.¹⁴ The application of VEGF for therapeutic neovascularization mobilizes the CPE.¹⁵

The first report of a case of the use of bone marrow stem cells (BMSC) was carried out by Dr. Bodum Strauer in August 2001, who safely carried out the injection of BMSC via coronary in a patient after IAM. Later Hamano et al. reported five cases carried out in Japan, where the intramyocardial transplant of the BMSC was carried out during revascularization surgery, where three of the five patients showed improved myocardial perfusion in the injected areas.¹⁶

Infusion of the gene of fibroblast growth factor (FGF) was carried out via intracoronary, using adenovirus as a vector (Ad5-FGF5) in 79 patients (19 received placebo), with symptom and contractility improvement in the treated group.¹⁷ Later in the same year another study was published: randomized, double blind, with placebo, with a scaled dose of phVEGF in 18 patients and placebo in nine patients, subjected to transendocardial injections, also via NOGA injection catheter.¹³

These preliminary studies in animals and clinical tests have two implications: first, they suggest that the fundamental mechanism by which neovascularization increases the network of collaterals is through the supply of supplementary cytokine to individuals who, due to their advanced age, diabetes, hypercholesterolemia and other still undefined circumstances, are unable to increase its supply in response to tissue ischemia. Recently, Heeschen et al. have shown that the ability to recruit stem cells from the bone marrow may be impaired in certain older adults.¹⁸ Second, administering cytokine clearly represents only one factor of the therapeutic intervention. Independently of how much is administered, the population of resident endothelial cells capable of responding to a certain level of vascular growth factor can constitute a potential limiting factor for the strategies designed to promote neovascularization in ischemic tissues.

The therapeutic angiogenesis with recombinant fibroblast growth factor-2 for intermittent claudication (TRAFFIC) was the first clinical randomized study controlled by placebo that showed a positive effect of the treatment with fibroblast growth factor (FGF-2) in 180 patients with critical limb ischemia.¹⁹ Their hypothesis was that intra-arterial rFGF-2 increases exercise capacity in patients with moderate to severe intermittent claudication due to infrainguinal peripheral artery disease.

To induce angiogenesis, investigators have delivered VEGF and basic fibroblast growth factor (bFGF), or hypoxia – inducible factor. In this study, it has become clear that the subsequent stage of remodeling and stabilization are crucial for attaining stable and functional vessels.²⁰

Iba et al.²⁰ clearly showed that intramuscular implantation of human peripheral blood mononuclear cells and platelets into ischemic limbs effectively induces collateral vessel formation mainly by supplying VEGF.

Al-Khaldi et al. evaluated the effect of local autologous bone marrow stromal cells to induce a neovascular response, after ligating the left common iliac artery of male Lewis rats, resulting in a significant increase in blood flow to the ischemic limb. Also, the ability of spontaneous regeneration of muscular tissues.²¹

Cell-based therapy has the advantage that the implantation of a single agent may result in the sustained production of multiple arteriogenic growth factors.²² Tateishi-Yuyama et al., in a clinical study using bone marrow cells, showed a sustained arteriogenic response with cell-based therapy.²³

Furthermore, it opened the way to the discovery that there is vasculogenesis in adult life, i.e., that new vessels appear in adult life, not only the concept of replication of capillaries from existing vessels called angiogenesis. After its publication, others followed confirming these results.^{8,24}

Recently it was demonstrated that the freeing of cytokines as VEGF and bFGF modulated by bone marrow cells remodel collateral circulation more actively than by direct cell incorporation. The spectrum of the genic expression of the cytokines by bone marrow cells is related to the paracrine mechanisms that support the biological effects of cell therapy for ischemic tissue.

This work showed that, although there was some degree of revascularization in the control group, the repeated injection of the conditioned medium induced a much more efficient angiogenic response.

This group admits that freeing the cytokines is sufficient to mediate arteriogenesis and collateral circulation after cell therapy. However, complementary mechanisms must contribute to the beneficial effect and the formation of blood vessels. The importance of the mechanism is still unknown.²⁴

The first study during a myocardial revascularization surgery in humans was carried out with five patients, injecting stem cells in non-revascularizable areas of the myocardium. The results showed that three of the five patients treated in that manner improved the myocardial perfusion after a year of following their development. Also, this confirmed the results of previous experimental studies in animal models, showing that there were no harmful alterations in the hearts where the stem cells were injected.

In the TOPCARE study, carried out in Germany, the BMSC (n = 9) and the SC obtained from peripheral blood and expanded *in vitro* in culture (n = 11) were administered by intracoronary infusion 4 days after the IAM in patients that had been subjected to primary angioplasty.²⁵ In Brazil research involving stem cells has seen important advances. The partnership of the *Hospital Pró-Cardíaco* of Rio de Janeiro with the Texas Heart Institute and the *Universidade Federal do Rio de Janeiro*, made possible a study with transendocardial implantation by catheter of BMSC in patients with severe ischemic cardiopathy without the possibility of conventional myocardial revascularization.²⁶

Fourteen patients were subjected to cellular therapy. The transplants were made with the use of NOGA catheters, and the procedures were carried out without major complications, so that all patients were discharged in 48 hours.

In a 2-month follow-up there was a significant improvement of the symptoms. Improvement of myocardial perfusion, with a reduction in the ischemic area from 15.1 to 4.5% in the LV (left ventricle) ($P = 0.02$), reduction in the final systolic volume of 15% ($P = 0.03$) and relative improvement of 31% in the FE ($P = 0.0004$).

On August 2002 the first clinical study in humans was published, the autologous transplant of bone marrow mononuclear cells in 52 patients with chronic limb ischemia, through injections in the sural region, based on previous and experimental models of limb ischemia and myocardial ischemia.²³

They noted that CD31+ endothelial cells express ki-67 in the marrow-implanted limb. Ki is a nuclear protein that is expressed in proliferating cells and is scarce in normal vessels.^{23,27} The safety of the method and its effectiveness in developing therapeutic angiogenesis has been demonstrated, probably related to the presence of endothelial progenitor cells in the bone marrow, and the possibilities of such cells secreting cytokines that stimulate angiogenesis.

This group treated 45 lower limbs with MBMC and observed significant improvement in the ABI, TCO², increase in painless walking time, increase in the flow with laser Doppler and formation of vessels in arteriography.

Implantation of MBMC improved pain at rest in these patients and the healing of ischemic wounds, demonstrating the efficiency of the procedure.²³

Conclusions

Although both surgical bypass and endovascular procedures remains effective in the improvement of the blood flow in the ischemic legs, not all patients are candidates for intervention. The effort in basic science laboratories has showed us the safety of the therapeutic angiogenesis, but we need to wait for more randomized trials in order to draw further conclusions.

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