Review Article

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Prospects of Adult Stem cells therapy in Peripheral Vascular Diseases

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ABSTRACT

Peripheral Vascular Disease (PVD) is a growing medical problem and presents itself mainly in two different clinical forms. Intermittent claudication is an early moderate manifestation, while patients with critical limb ischaemia suffer from severe muscle tissue loss or ulcers and are at high risk of limb amputation. Despite recent advances in surgical and radiologic vascular procedures, a large number of patients are not eligible for these revascularisation procedures. Recent evidence indicates that adult stem cells (ASC) are a potential new therapeutic target. This review discusses the potential of ASC in patients with PVD. The safety of stem cells must be scrutinised and assessed throughout the entire treatment and research process. Guidelines and strategies must also be developed to ensure that every aspect of stem cell use from identification and isolation of stem cells to stem cell transplant is stringently coordinated.

KEYWORDS

Adult stem cells, peripheral vascular disease, critical limb ischaemia, therapeutic neo-angiogenesis.

ABBREVIATIONS

Peripheral Vascular Disease (PVD),Adult Stem Cell (ASC),Intermittent Claudication (IC),Critical Limb Ischemia (CLI),Peripheral Arterial Occlusive Disease (PAOD), Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factors (FGFs),Bone Marrow (BM), Endothelial Progenitor Cells (EPC), Bone Marrow Mononuclear cells (BM-MNC), Granulocyte Colony Stimulating Factor (G-CSF),Peripheral Blood Mononuclear Cells (PB-MNC), Therapeutic Angiogenesis using Cell Transplantation (TACT).

Introduction

Currently, peripheral vascular disease (PVD), causing an inadequate oxygen supply to the limbs, globally affects no less than 3–10% of the population[1]. Peripheral vascular disease, including diabetic foot, arteriosclerosis obliterans, and thromboangitis obliterans, commonly affect the arteries supplying the leg. Based on the severity of the symptoms, usually two clinical presentations are distinguished: intermittent claudication (IC) is characterised by pain upon walking while critical limb ischaemia (CLI) is a more severe form in which pain occurs at rest and which is accompanied by necrosis and ulceration.

Peripheral arterial occlusive disease (PAOD) is estimated to develop in 500 to 1000 individuals per million persons per year[2, 3]. The prevalence of all stages of PAOD in the general population is estimated to be 4.2% to 35%. Within this group, 4.3% to 9.6% will experience progression of the disease towards CLI, eventually resulting in amputation of the affected limb[4]. Diabetic PAOD patients are at the highest risk within this patient group: they are about 10 times more likely to come to amputation, and the prevalence of gangrene is 20 to 30 times higher[2]. CLI has important functional implications and a major impact on the quality of life. Quality of life indices of patients with CLI have been reported to be similar to those of terminal cancer patients[5]. In addition, CLI is associated with surgery and hospitalisation[6]. CLI is also associated with increased mortality (the 1-year mortality is approximately 25% and may be as high as 45% after amputation)[7] , and even asymptomatic PAOD by itself is a significant predictor of cardiovascular morbidity and death[8].While obstructive atherosclerotic disease is the most common cause of PVD, some forms of vasculitis, such as thromboangiitis obliterans or Buerger's disease, also result in peripheral ischaemia (in feet and/or hands), often progressing to tissue loss and major amputations[9,10].

Unfortunately, a significant proportion of patients (including both IC and CLI cases) are not eligible for or do not beneficially respond to these revascularisation procedures due to the widespread nature or the distal location of the obstructions or due to the presence of co-morbidities putting them at higher risk for peri-procedural death.

For these 'no-option' patients, non-invasive revascularisation strategies have been introduced, which fall into two categories: single gene/protein-based or cell-based strategies. Angiogenic growth factor (e.g., vascular endothelial growth factor (VEGF), fibroblast growth factors (FGFs), and hepatocyte growth factor) therapy has been tested clinically since more than 5 years. But the overall benefit for PVD patients has been disappointing[11].

Consequently, exploring new strategies for revascularisation of ischaemic limbs is of major importance.

What are stem cells?

Stem cells are defined as a cell population capable of self-renewal, proliferation and differentiation. They serve as a repair system for the body.

Stem cells are classified into two different types during the development of the organism: embryonic stem cells and adult stem cells (ASCs).

The use of adult stem cells in research and therapy is not as controversial as embryonic stem cells, because the production of ASC does not require the destruction of an embryo. Additionally, because in some instances ASC can be obtained from the intended recipient, (an autograft) the risk of rejection is essentially non-existent in these situations.

Where are adult stem cells found, and what do they normally do?

Adult stem cells (ASCs) have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. In HYPERLINK "http://en.wikipedia.org/wiki/Adult" \o "Adult"adult organisms, stem cells and HYPERLINK "http://en.wikipedia.org/wiki/Progenitor_cell" \o "Progenitor cell"progenitor cells act as a repair system for the body, replenishing specialised cells, but also maintain the normal turnover of regenerative organs, such as blood, skin or intestinal tissues. They are thought to reside in a specific area of each tissue (called a "stem cell niche"). In many tissues, current evidence suggests that some types of stem cells are pericytes, cells that compose the outermost layer of small blood vessels. Stem cells may remain quiescent (non-dividing) for long periods of time until they are activated by a normal need for more cells to maintain tissues, or by disease or tissue injury.

The concept of stem cell based revascularisation emerged in 1997, when Isner's group described circulating cells in adults called endothelial progenitor cells (EPC) with the capacity to differentiate into endothelial cells (EC) and incorporate into new vessels in ischaemic tissue[12]. Since then, the number of studies reporting on stem cell related revascularisation has exponentially increased. Bone marrow (BM) derived stem cells have been identified as a potential new therapeutic target. Most adult stem cells are lineage-restricted (multipotent) and are generally referred to by their tissue origin for example: mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell etc[13, 14].

In the 1950s, researchers discovered that the bone marrow contains at least two kinds of stem cells. One population, called hematopoietic stem cells, forms all the different types of blood cells in the body. A second population, called bone marrow stromal stem cells (also called mesenchymal stem cells, or skeletal stem cells by some), were discovered a few years later. These non-hematopoietic stem cells make up a small proportion of the stromal cell population in the bone marrow, and can generate bone, cartilage, fat, cells that support the formation of blood, and fibrous connective tissue.

Adult stem cell treatments have been successfully used for many years to treat leukaemia and related bone/blood cancers through bone marrow transplant[15].

Relationship between neoangiogenesis and cell population.

Neoangiogenesis:

Three concepts of vascular growth have been described to date—angiogenesis, vasculogenesis, and arteriogenesis (collateral artery growth)—which represent different aspects of an integrated process. Stimulation of arteriogenesis seems clinically most relevant and has most recently been attempted using autologous bone marrow transplantation with some beneficial results, although the mechanism of action is not completely understood.

Cell population:

Hematopoietic stem cells may be CD34+ AC133+ or CD34- AC133+ or CD34+ AC133-. Vascular development is regulated by growth factors and their receptors such as vascular endothelial growth factor (VEGF) and VEGF tyrosine kinase receptors such as VEGFR-1 (flt-1) or VEGFR-2 (KDR or flk-1). Other growth factors such as angiopoietin-1 that bind a tyrosine kinase receptor Tie-2 may be involved in completing the vascular architecture by assembling pericytes and smooth muscle cells around endothelial cells[16].

Marrow or peripheral blood CD34+ hematopoietic stem cells express VEGFR and Tie.12 When cultured ex-vivo in fibronectin-coated flasks with VEGF, CD34+ AC133+ cells differentiate into endothelial cells by morphology, acetylated low-density lipoprotein incorporation, nitric oxide release, Von Willebrand factor expression, and lectin binding[17].

The unfractionated mixture of hematopoietic mononuclear cells includes more differentiated cells that are thought to provide angiogenic cytokines as well as stem cells that become incorporated into collateral vessels by a process of neoangiogenesis. In clinical trials, Tateishi-Yuyama et al.[18] injected autologous bone marrow mononuclear cells into patients with ischaemic PVD. Patients were selected for chronic ischaemic extremity pain or non-healing ischaemic ulcers or both and a resting blood pressure ankle-brachial index less than 0.6. Bone marrow cells were collected under general anaesthesia from the posterior superior iliac crest and with a 26-gauge needle injected into the gastrocnemius muscle of the ischaemic leg in multiple sites divided by a 3x3 cm grid. Significant improvement in the ABI, trans-cutaneous oxygen pressure, and pain-free walking occurred following treatment[18].

Several independent clinical studies have reported beneficial effects of the administration of bone marrow mononuclear cells (BM-MNC), Granulocyte Colony Stimulating Factor (G-CSF) mobilised Peripheral Blood Mononuclear Cells (PB-MNC), G-CSF-mobilised PB-MNC after ex vivo culturing, G-CSF mobilised CD34+ cells, and G-CSF mobilised CD133+ cells in patients with CLI. However, no direct comparisons have been performed and it is still unclear which cell types or subpopulations provide the best treatment results. The progenitor cells specifically involved in vascular repair and neovascularisation were initially thought to originate from the CD34+ hematopoietic progenitor cell population, analogous to the common hemangioblast precursor in embryonic development[19, 20].

Consistently, in the Therapeutic Angiogenesis using Cell Transplantation (TACT) study, legs that were injected with PB-MNC, containing approximately 500-fold less CD34+ cells than BM-MNC, showed much smaller increases in collateral perfusion as compared with BM-MNC-injected legs.[18,21] Furthermore, Saigawa et al demonstrated a correlation between the number of implanted CD34+ cells and the efficacy of bone marrow implantation[21].

However, several studies suggest that CD34- cell populations also play an important role in the beneficial effects of BM cell therapy. Asahara et al already showed that CD34- cells, added to CD34+ cells in culture, improved outgrowth of cells with an endothelial phenotype[12]. Co-culture of CD34+ cells with CD34-cells in an in-vitro 3-D matrix model using human microvascular endothelial cells significantly enhanced neovascularisation as compared with CD34+ cells alone[22].Other groups described that non-hematopoietic bone marrow mesenchymal precursor cells and myeloid/monocyte lineage cells (CD14+) can also differentiate into EPC or into cells with EPC characteristics[23-26]. Iba et al compared the angiogenic effects of the same numbers of BM-MNC and PB-MNC (containing 2.4% and 0.02% CD34+ cells, respectively) in a rat hind limb ischaemia model and showed that although there was no incorporation of PB-MNC, the angiogenic effect of PB-MNC was approximately 72% relative to that of BM-MNC[27]. Moreover, Tateno et al showed that there was no significant difference in stimulation of neovascularisation after infusion of PB-MNC and BM-MNC[10].

These data suggest that, apart from incorporation of EPC, EPC supply of angiogenic factors such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and angiopoietin-1 plays an important role. This role of the paracrine effects of EPC on vascular growth have also been demonstrated by the group of Schaper[28, 29].

A recent report proposed that implanted cells stimulate muscle cells to produce angiogenic factors, thereby promoting neovascularisation[10]. Yang and co-workers reported a simple and effective therapeutic approach for diabetic limb ischaemia by autologous transplantation of G-CSF -mobilised peripheral blood stem cells[30].

Thus, different cell populations are involved in vascular repair and neovascularisation, and these cells may act via direct incorporation into the endothelial layer and endothelial differentiation, by supply of angiogenic factors, or by a combination of both[31].

The majority of studies on cell therapy for CLI have used whole MNC fractions and at this moment it is unclear whether administration of more selected cell populations or ex-vivo culture toward an endothelial phenotype would be more effective.

Although clinical studies showed promising results from both BM-MNC and G-CSF-mobilized PB-MNC, recent data suggest that functional activity of the G-CSF mobilised cells, as assessed by the migratory response to VEGF and stromal cell-derived factor1, is significantly reduced as compared with non-mobilised cells from the same patient. Also in in-vivo experiments in nude mice with hind limb ischaemia, G-CSF-mobilised EPC show a reduced capacity to augment blood flow recovery and to prevent necrosis as compared with the same EPC without G-CSF stimulation[32].

It is important to note that cell isolation protocols may also have a major impact on the functional activity of BM-derived progenitor cells[33].

Optimal Dosage

It is remarkable that all studies discussed above reportfavourable outcome, despite varying dosages, with an even so varying concentration of $CD34^+$ cells. In the studies involving BM cell administration, amounts of aspiratedBM cell ranging from 80 to 1000 ml, from which theinjected dosage of progenitor cells was retrieved, were reported.In the TACT Study[18] and in the study by Higashi etal.[34] approximately 1.6x 10^9 MNC were obtained from 500ml of BM, whereas Durdu et al.[9] retrieved a 50-fold of MNCfrom the same amount of BM (101x10^9 MNC from 653 mlof BM). Bartsch et al.[35]separated a 2.5 times smaller amountof MNC from the same amount of BM (0.1x10^9 MNC from80 ml of BM). The fraction of $CD34^+$ cells in the isolatedMNC population varies from 0.6% in the study by Kajiguchiet al.[36] to 2.4% in the TACT study[18].

Clinical Evaluation

Currently used measures for clinical evaluation, such as ankle-brachial pressure index, are subject to factors other than improvements in perfusion alone. In accordance with the Trans-Atlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC-II) recommendations, future trials should ideally combine multiple measures for clinical improvement and quantification of the arterial flow to evaluate treatment success, which include ankle pressure, toe pressures,

TcPO2, microcirculation investigation methods like laser Doppler fluxometry, and anatomic imaging[1].

In addition, questionnaires addressing pain experience, pain "magnitude" (pain intensity, emotion, cognitive-evaluative, and sensitivity) and pain at rest (on a visual analogue scale), as well as quality of life questionnaires will provide patient-based parameters for the clinical effects of therapy.

Ulcer status should be assessed by measurement of the cumulative total ulcer area, with ulcer healing defined as healing of all ulcers of the treated leg. Limb status can be assessed using the criteria of Rutherford[37].

Contrast-enhanced high spatial resolution magnetic resonance angiography is a reproducible and robust modality for assessment and quantification of new vessel formation, detecting different sizes of collateral vessels, and determination of (changes in) tissue perfusion.

However, Choksy and Chan[38] pointed out that a major scientific weakness in angiogenesis research lies in the assessment of vascular growth.

Avenues to explore?

- How do adult stem cells evolve during development and how are they maintained in the adult? Are they "leftover" embryonic stem cells, or do they arise in some other way?
- If the beneficial effect of adult stem cell transplantation is a trophic effect, what are the mechanisms?
- What are the factors that control adult stem cell proliferation and differentiation?
- What are the factors that stimulate stem cells to relocate to sites of injury or damage, and how can this process be enhanced for better healing in PVD?
- Why do stem cells remain in an undifferentiated state when all the cells around them have differentiated? What are the characteristics of their "niche" that controls their behaviour?
- How can assessment of neo-angiogenesis be improved?

Conclusion

Clearly, stem cell safety must be scrutinised and assessed throughout the entire treatment or research process. Guidelines and strategies must also be developed to ensure that every aspect of stem cell use - from identification and isolation of stem cells to stem cell transplant - is stringently coordinated.

Although several clinical studies show promising results, larger randomised, blinded, placebo-controlled trials are needed to provide definite proof of the clinical effects of adult stem cell therapy in these patients. In addition, questions regarding the cell population(s) to be used, optimal dose, and routes of administration will have to be addressed. If doctors and scientists can establish safe protocols for stem cell use, everyone can benefit from the full potential of the remarkable and possibly life-saving stem cell therapies.

Competing Interests
None declared
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