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REVIEW ARTICLE

Stem cell therapy for critical limb ischaemia – a review

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Abstract Critical limb ischaemia is an intractable condition associated with high levels of amputation, leading to a low quality of life and increased morbidity and mortality. It is often not treatable by standard therapeutic modalities. Neoangiogenesis has been proposed as a novel method of treatment of such patients. Vascular endothelial growth factor (VEGF) and cytokine fibroblast growth factor (FGF-1) have been shown to elicit neoangiogenesis. Stem cells are progenitor cells which can differentiate *in vivo* into different types of cells. Mesenchymal stem cells (MSCs) are a type of adult stem cells which have an immunomodulatory effect. Stem cell therapy has been used in animal studies to improve limb vascularity in rat and rabbit models. Several clinical studies have also validated their use for critical limb ischaemia. However many issues are still unresolved. These include the dosage, delivery and safety issues in relation to stem cell therapy. However stem cells are likely to be an important therapeutic modality to treat critical limb ischaemia in the near future.

Keywords Stem cells · Critical limb ischaemia · Neoangiogenesis

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Introduction

Critical limb ischaemia (CLI) is a vexing problem for all clinicians. It has been defined as a condition where there is rest pain, ulcers and gangrene in a setting of proven occlusive arterial disease [1]. It is common in out patients' departments to see a smoker who has presented with severe foot pain, an ulcer or a gangrenous toe. Such patients are very difficult to treat and can tax the clinician as they are refractory to all conventional forms of treatment. Despite all treatment by advanced modes, including surgical and interventional radiologic interventions, a substantial number of patents will need a major or minor amputation.

The diagnosis of critical limb ischaemia is made clinically by the characteristic symptomatology and the lack of foot pulses. This is backed up by investigations which include ankle brachial index (ABI) (<60%), low ankle pressures (<50 mmHg), reduced transcutaneous oxygen (TPC O_2) (less than 30–50 mmHg). The blockage is demonstrated by arterial Doppler examination and angiography.

The condition is extremely common. It is estimated that as many as 20% of Americans ≥65 years old and 50% ≥75 years old have peripheral arterial disease (PAD) [2]. In Britain, the Vascular Society found a true amputation rate of 21.5% [3]. Another study claims that it may affect 5% of Americans above the age of 40 and this rises to 12–14% in patients who are older than 70 years. Thus a staggering 8 million Americans are estimated to suffer from limb ischaemia [4]. Incidence levels in India are not clear but the disease load is likely to be at least twice or thrice this number. Limb ischaemia is associated with major levels of morbidity and mortality. As many as 10–40% of the patient population undergoes an amputation sometime during the course of the illness and the one year mortality has been estimated

to be as high as 45% in these patients [5]. The quality of life in these patients have been described to be similar to those of terminal cancer patients [6]. Diabetes, if present makes a bleak situation even worse. Diabetics are ten times more likely to have an amputation and 20–30 times more likely to develop gangrene [7].

Options for patients with critical limb ischaemia are limited and less than half these patients are eligible for the surgical or percutaneous intervention. As one paper puts it "Critical limb ischaemia: nothing to give at the doctor's office" [8].

Angiogenesis therapy is likely to be a method that will offer new hope to these patients. The use of stem cells to produce new vascularisation may be the way forward for this group of patients.

Angiogenesis

It has long been hoped that neoangiogenesis, stimulated by artificial means will be the way out for this seemingly insurmountable problem. It is known that this can act as a biological bypass to produce new collaterals which will restore blood supply to the muscles that are affected. It has been long noted that ischaemic muscles secrete angiogenic factors in response to hypoxia which leads to new vessel formation [9]. This has been proved in animal models. One such factor is the vascular endothelial growth factor (VGEF). Another such factor is the cytokine fibroblast growth factor (FGF-1). In animal studies it has been shown that while VGEF is associated with leaky blood vessels, FGF results in creation of more mature vessels and may be ideal for new blood vessel formation [10].

The use of stem cells for this purpose has two advantages. Not only does it differentiate into endothelium, thus increasing the number of new cells, it also produces various growth factors capable of stimulating angiogenesis, like FGF and VGEF.

Stem cells

Stem cells are cells which have three basic properties. They are unspecialised cells, capable of replicating themselves, and can differentiate into different types of specialised cells. Stem cells retain the ability to become many or all of the different cell types in the body and thereby play a critical role in repairing organs and body tissues throughout life. There are two types of stem cells, embryonic which are derived from an early embryo and adult stem cells which are obtained from various body tissues including adipose tissue, skin, dental pulp and most commonly from the bone marrow.

Embryonic stem cells are pluripotent, (that is, they can produce cell types from all three embryonic germ layers: mesoderm, endoderm, and ectoderm), self-renewing cells derived from the inner cell mass of blastocyst stage embryos, while adult cells can differentiate into cells of their tissue of origin. Embryonic stem cells have raised many ethical issues; also they are often associated with tumourogenesis and thus are not widely used, at least yet for therapy. Embryonic stem cells were derived by the University of Wisconsin group from 1-week-old embryos produced by *in vitro* fertilisation. Another possibility is to produce stem cells by somatic cell nuclear transfer, also known as cloning [11].

Adult stem cells again can be of haeamatopoeic or mesenchymal origin. They are ubiquitous and during life act as a reservoir to replace worn out or damaged cells. Of the adult stem cells, mesenchymal stem cells have become the focus of most attention. They have two great advantages which are very important for therapeutic use. One is the property of mesenchymal cells of not expressing many of the CD proteins: this leads to a protection from immune effects of the host. Not only that they also secrete some bioactive factors which give them an immunomodulatory function. This is important because it raises the possibility of allogenic transplants which is an important advance [12].

No matter what their origin, stem cells act by two mechanisms. First, they directly replace the affected cells by differentiating into the type of cells that are damaged. Secondly they have paracrine effects by secretion of growth factors to stimulate local stem cell growth or by signals which recruit stem cells from elsewhere or by modulating the immune system [13].

Cytokine mediated angiogenesis for CLI

The concept of therapeutic angiogenesis was first proposed by Folkman in 1971 when he suggested that angiogenic factors were responsible for maintaining tumour blood supply [14]. It was realised later that the same mechanisms could be used to regenerate vascularity in a clinical situation where there was ischaemia, a sort of biological bypass as it were. In animal experiments, femoral artery ligated rat models injected with VGEF did not undergo limb necrosis as did control animals. Several studies [15, 16] subsequently showed the efficacy of this approach to promote neoangiogenesis as well as paracrine effects to prevent ischaemia. Unfortunately double-blind clinical studies did not show any advantage of this therapy. Not only that some concerns were raised about the development of "leaky" blood vessels due to the immaturity of the newly developed blood vessels.

This led to the use of the FGF-1 which is a precursor of the VGEF. It was postulated that this would lead to creation of more mature blood vessels which would not "leak". The difference between tumour blood vessels and non-tumour angiogenesis is the ratio between VGF and FGF-1, the increased levels of FGF-1 leading to more mature blood vessel formation in normal healing processes [17]. This

process has been the subject of clinical trials which have shown good results, but the principal problem has remained the lack of well controlled, adequately powered double-blind controlled trials. This approach remains promising, but much work needs to be done before it can be proposed as a reasonable alternative in clinical practice.

Stem cell treatment in Critical limb ischaemia

The use of stem cells is based on the premise that the delivery of stem cells to the ischaemic tissue will lead to the development of new blood vessels. This occurs because the cells differentiate into endothelial cells as well as because of the paracrine effects of these cells which promote secretion of factors which recruit *in vivo* stem cells. This concept was also tested by rat models in the laboratory. Several studies demonstrated that rats, whose femoral arteries had been ligated, developed new blood vessels when they were injected with stem cells. Not only that they did better in treadmill studies and also showed increased perfusion of the ischaemic limbs. In one early study using recombinant protein, a single intra-arterial injection of 500–1000 μg VEGF165 into rabbits with severe experimental hind limb ischaemia increased collateral vessels, as detected by angiography and histological analysis [18]. However it must be noted that animal models do not replicate the human clinical condition because the ischaemia produced experimentally is acute while critical limb ischaemia is a final stage of a long standing and progressive disease.

This led logically to the use of stem cells in clinical practice. Bone marrow transfusion has been in use for clinical purposes for the past 40 years and is well accepted for many uses in haematological practice. Tateishi-Yuyama and his group published a paper in 2002 [19] where they showed that they could prepare stem cells from an autologous source. This was used for CLI treatment by injecting the cells into the gastrocnemius muscle. This led to a statistically significant increase of ABI, TCO_2 and claudication distance. A recent review of clinical studies [20] found that 25 clinical studies had been published so far. Of these, 18 studies in English, were analysed and found to have promising results. However there are a host of unanswered issues which include the dosage, optimal route of administration and the need, or otherwise of multiple injections. There is also a lack of consensus about the correct method of proving the neoangiogenesis. It is generally accepted that contrast enhanced high spatial resolution magnetic resonance angiography is a robust means of assessment of new vessel formation and development of collateral circulation.

However there is a big drawback in the use of autologous cells for the purpose of treating CLI. The patient population usually has several co-morbidities and patients are often seriously ill. Under such conditions, anaesthesia for bone marrow aspiration and a long wait for about a month to grow the cells is not a practical proposition. The patient needs help fast and without any intervention that might add to his morbidity. This has led to the popularity of another approach, the use of mesenchymal stem cells as an allogenic transplant. The idea is to have bone marrow from healthy donors and culture them in the lab to have cells available "off the shelf" for use as and when needed. As discussed earlier MSCs are ideally suited for this purpose because they are non-immunogenic and can be used as an allogenic transplant without any danger of rejection. MSCs have successfully passed Phase I and Phase II trials in the USA for use in Crohn's disease and Graft vs Host disease and are in advanced stages of a Phase III trial. Several Indian companies are also in the process of developing such products for off the shelf use. It is now a subject of an ICMR approved clinical trial in to prove its efficacy and safety; the preliminary results should be available in about a year's time.

Questions have been raised on the efficacy of progenitor cell therapy in diabetes which is a major accompanying morbidity in many cases of CLI. Several animal studies have addressed this question. They have been clear indications that ischaemia caused or aggravated by diabetes have been improved by stem cells [21, 22].

Anecdotal evidence exists of the efficacy of stem cell therapy in the diabetic foot. It remains for a clinical trial to substantiate this effect. Intuitively however there is no reason to believe that progenitor cell therapy will not be as effective in diabetics as in non diabetics. Mesenchymal stem cells have been used as a mode of therapy for type I diabetes mellitus with success [23] and thus would probably be as effective in limb ischaemia associated with diabetes.

Indications for stem cell therapy

The principal indications that stem cell therapy can address are:

1) Thromboangitis obliterans (TAO)

TAO is a disease that is exceptional in that it obliterates distal arterial beds. In such patients distal arterial obstruction may obviate successful revascularisation and even if it were possible patency would not be maintained. Such patients are likely candidates for stem cell therapy and a trial has shown its efficacy for this indication [24]. This study had a fairly long follow up of upto 4 years and objective as well as subjective criteria proved the efficacy of stem cells.

2) Atherosclerotic disease

Other studies have shown the efficacy of progenitor cells in atherosclerotic disease affecting the infrapopliteal region which is well nigh untreatable by conventional means [25]. The authors demonstrated marked increase in walking

distance, ankle brachial index and flow dependent vasodilatation after the injection of stem cells via the intra-arterial route.

Delivery of stem cells

The standard method used by most investigators has been to deliver the cells by direct injection into the gastrocnemius muscle. The idea is to build up a local depot of stem cells which will augment vascularisation by any of the three methods discussed earlier. Other methods used have been intra-arterial injection or a combination of both.

The usual practice is to use 2 million cells/kg body weight in 0.5 ml/kg of the cell suspension ml. For a 50 kg patient this means 100 million cells in 25 ml of suspended solution. A grid map is drawn on the gastrocnemius, measuring 10 × 60 mm. Of the 60 grid areas produced the drug is injected into 40–60 areas at about 0.5 ml in each injection. The patient is admitted for one day and released after 24 hours of observation.

Intra-arterial injection deposits the stem cells to the border of the ischaemic zone [26]. Several studies have investigated the use of intra-arterial injections [25, 27, 28].

Is stem cell therapy safe?

As Hippocrates pointed out long ago, it is incumbent on clinicians to ensure "Primum non nocere—above all do no harm". It is essential to be sure that these novel methods of therapy are safe for the patient. There are two principal concerns. One is the possibility of tumourogeneis by the stem cell therapy. As these stem cells are associated with neovascularisation in tumours, it is logical to think that they may produce tumours in subjects being treated with these endothelial progenitor cells. Another concern has been the possibility of these cells destabilising atherosclerotic plaques and accelerating the disease process. Fortunately both these apprehensions were found to be unfounded in clinical studies done so far. The problem that does occur is a transient hypotension during injection and this has never proved to be of clinical significance.

Future directions in research

There are many gray issues to be sorted out before the use of stem cells can become a standard method of therapy. There is as yet no major double-blind randomised clinical trial of adequate power to prove beyond doubt the efficacy of this form of treatment. It is essential now for groups working in these areas to organise one such trial that will put the efficacy of stem cells beyond any doubt. There is also the issue of delivery. It is quite apparent now that no one method of delivery will suit all diseases, there is no "one size" that "fits all". What is the optimum dose and how many doses are needed? Guidelines about the manufacturing, storage and delivery of these cells have to be formalised and accepted by the bio medical community and the regulatory bodies. The use of allogenic material also needs to be validated and standardised. There are many issues including the shipping of allogenic cells, their quality control and proper documentation which need to be sorted out.

Conclusion

Stem cell therapy is likely to be a novel and successful therapy for CLI. All preclinical, animal studies as well as clinical trials have so far been successful. However general clinical use is still at least 2–3 years away. The use of neoangiogenesis therapy promises a biological cure for a biological problem, and this can be tailored for the specific patient population. Thus it is likely to be the answer for an intractable problem which has been a major cause of morbidity and mortality in patients with lower limb ischaemia.

Conflict of interest The authors do not have any disclosable interest

References

1. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG (2007) Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II). Eur J Vasc Endovasc Surg 33:S1–S75
2. Hirsch AT, Criqui MH, Treat-Jacobson D, et al. (2001) Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA 286:1317–1324
3. Anon (1996) Critical Limb Ischaemia: management and outcome: Report of a National Survey: The Vascular Society of Great Britain and Ireland. Eur J Endovac Surg 12: 131–135
4. Anon (2005) Lower extremity disease among persons aged > or=40 years with and without diabetes—United States, 1999–2002. Centers for Disease Control and Prevention (CDC). MMWR Morb Mortal Wkly Rep 54: 1158–1160
5. Dormandy JA, Rutherford RB (2000) Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Consensus (TASC). J Vasc Surg 31:S1-S296
6. Albers M, Fratezi AC, De LN (1992) Assessment of quality of life of patients with severe ischaemia as a result of infrainguinal arterial occlusive disease. J Vasc Surg 6:54–59
7. Dormandy J, Heeck L, Vig S (1999) Predicting which patients will develop chronic critical leg ischaemia. Semin Vasc Surg 12:138–141
8. Schainfeld RM, Isner JM (1999) Critical limb ischaemia: nothing to give at the office? Ann Intern Med 130:442–444

9. Milkiewicz M, Pugh CW, Egginton S (2004) Inhibition of endogenous HIF inactivation induces angiogenesis in ischaemic skeletal muscles of mice. J Physiol 560:21–26
10. El Oakley RM, Seow KK, Tang TP, Kok CW, Teh M, Lim YT, Lim SK (2002) Whole bone marrow transplantation induces angiogenesis following acute ischaemia. Redox Rep 7:215–218
11. Asahara T, Murohara T, Sullivan A, et al. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. Science 275:964–967
12. Ippokratis P, Giannoudis P V (2005) Biology of mesenchymal stem cells injury. Int J Care Injured 36S:S8–S12
13. Gerwins P, Skoldenberg E, Claesson-Welsh L (2000) Function of fibroblast growth factors and vascular endothelial growth factors and their receptors in angiogenesis. Crit Rev Oncol Hematol 34:185–194
14. Folkman J (1971) Tumor angiogenesis: therapeutic implications. N Engl J Med 285:1182–1186
15. Pu LQ, Sniderman AD, Brassard R, Lachapelle KJ, Graham AM, Lisbona R, Symes JF (1993) Enhanced revascularization of the ischemic limb by angiogenic therapy. Circulation 88:208–215
16. van Weel V, Deckers MM, Grimbergen JM, van Leuven KJ, Lardenoye JH, Schlingemann RO, van Nieuw Amerongen GP, van Bockel JH, van Hinsbergh VW, Quax PH (2004) Vascular endothelial growth factor overexpression in ischemic skeletal muscle enhances myoglobin expression in vivo. Circ Res 95:58–66
17. McDonnell K, Bowden ET, Cabal-Manzano R, Hoxter B, Riegel AT, Wellstein A (2005) Vascular leakage in chick embryos after expression of a secreted binding protein for fibroblast growth factors. Lab Invest 85:747–755
18. Hirata K, Li Tao-Shang, Nishida M, Ito H, Matsuzaki M, Kasaoka S, Harmano K (2003) Autologous bone marrow cell implantation as therapeutic angiogenesis for ischemic hindlimb in diabetic rat model. Am J Heart Care Physiol 284:66–70
19. Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, Amano K, Kishimoto Y, Yoshimoto K, Akashi H (2002) Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. Lancet 360:427–435
20. Sprengers RW, Lips DL, Moll FL, Verhaar MC (2008) Progenitor cell therapy in patients with critical limb ischaemia without surgical options. Ann Surg 247:411–420
21. Shibata T, Naruse K, Kamiya H, Kozakae M, Kondo M, Yasuda Y, Nakamura N, Ota K, Tosaki T, Matsuki T, et al. (2008) Transplantation of bone marrow-derived mesenchymal stem cells improves diabetic polyneuropathy in rats diabetes 57(11):3099–3107
22. Waters RE, Terjung RL, Peters KG, Annex BH (2004) Preclinical models of human peripheral arterial occlusive disease: implications for investigation of therapeutic agents. J Appl Physiol 97(2):773–780
23. Hori Y, Gu X, Xie X, Kim SK (2005) Differentiation of insulin-producing cells from human neural progenitor cells. PLoS Med 2:e103
24. Kajiguchi M, Kondo T, Izawa H, Kobayashi M, Yamamoto K, Shintani S, Numaguchi Y, Naoe T, Takamatsu J, Komori K, Murohara T (2007) Safety and efficacy of autologous progenitor cell transplantation for therapeutic angiogenesis in patients with critical limb ischaemia. Circ J 71:196–201
25. Lenk K, Adams V, Lurz P, Erbs S, Linke A, Gielen S, Schmidt A, Scheinert D, Biamino G, Emmrich F, Schuler G, Hambrecht R (2005) Therapeutical potential of blood-derived progenitor cells in patients with peripheral arterial occlusive disease and critical limb ischaemia. Eur Heart J 26:1903–1909
26. Bartsch T, Brehm M, Zeus T, et al. (2006) Autologous mononuclear stem cell transplantation in patients with peripheral occlusive arterial disease. Cardiovasc Nurs 21:430–432
27. Bartsch T, Brehm M, Zeus T, Kögler G, Wernet P, Strauer BE (2007) Transplantation of autologous mononuclear bone marrow stem cells in patients with peripheral arterial disease (The TAM-PAD study). Clin Res Cardiol 96(12):891–899
28. Kim D-I, Kim M-J, Joh J-H, Shin S-W, Do Y-S, Moon J-Y, Kim N-R, Lim J-E, Kim A-K, Eo H-S, Kim B-S, Cho S-W, Yang S-H, Park C-J, Shim J-S (2006) Angiogenesis facilitated by autologous whole bone marrow stem cell transplantation for Buerger's disease. Stem Cells 24: 1194–1200