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Current Status of Stem Cell Therapy for Thromboangiitis Obliterans (Buerger's Disease)

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Abstract

Buerger's disease or Thromboangiitis Obliterans (TAO) is a nonatherosclerotic segmental vascular disease which affects small and medium arteries and veins in the upper and lower extremities. Based on pathological findings, TAO can be considered as a distinct form of vasculitis that is most prevalent in young male smokers. There is no definitive cure for this disease as therapeutic modalities are limited in number and efficacy. Surgical bypass has limited utility and 24% of patients will ultimately require amputation. Recently, studies have shown that therapeutic angiogenesis and immunomodulatory approaches through the delivery of cells to target tissues are potential options for ischemic lesion treatment. In this review, we summarize the current knowledge of TAO treatment and provide an overview of stem cell-based treatment modalities.

Key words: Thromboangiitis Obliterans; Inflammation; Angiogenesis; Immunomodulation; Pluripotent stem cell; Mesenchymal stem cell

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Introduction

Thromboangiitis Obliterans (TAO) is caused by vasculitis and inflammation of the peripheral blood vessels leading to impaired blood circulation, coagulation, and critical limb ischemia (CLI) [1]. TAO has a global distribution but is most prevalent in Middle and Far Eastern nationalities. Although the precise etiology still remains unknown [1], it predominantly affects young male and female smokers [2-4][5]. Just less than 5% of cases might be caused by other non-smoking related factors such as frostbite, extremity trauma, or even sympathomimetic drug abuse [3, 4, 6, 7].

TAO is a rare form of vasculitis which is distinguished from other types by cellular inflammatory thrombus formation with relative sparing of the vessel wall. TAO has also shown to contain elevated level of pro- and anti-inflammatory cytokines and autoantibodies [8-10]. While the precise pathophysiology behind TAO is controversial, it is believed to be due to an IL-33-mediated immune response resulting in vascular abnormalities [8, 11].
The clinical pathogenesis of TAO begins with lower extremity pain during physical activity which progresses to pain while resting. Additionally, patients may experience Raynaud's phenomena. Many patients also develop ischemic ulcerations that progress to gangrene [2-4]. Despite considerable advances in treatment options, TAO is still associated with high morbidity [12]. While pharmacological approaches and surgical intervention remain generally palliative, novel therapeutic approaches such as gene and stem cell therapy to promote angiogenesis have been considered promising for the treatment of TAO [13-16]. The chief aim of this paper is to provide an overview of the current treatment modalities of TAO and outline approaches using stem cell therapy that may provide new therapeutic solutions to halt the pathogenesis of TAO.

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Conventional Approaches

All patients with TAO are advised to stop smoking and avoid second-hand smoke exposure. However, cessation does not completely prevent disease development and progression [17]. Proper foot care is essential to monitor for and treat ischemic ulceration. Often emollient skin cream is helpful to prevent fissure formation.

Pharmacologic treatment:

Pharmacological treatment of TAO is focused on anticoagulation (Aspirin), vasodilators (calcium channel blockers), systemic anti-inflammatory drugs (prostacyclins analogs) and analgesics. [18, 19] Previous studies have demonstrated that iloprost is effective for analgesia and improved wound healing potential. These properties are superior with intravenous infusion. Additionally, thrombolytic therapy with streptokinase and urokinase have demonstrated utility for the treatment of toe and foot gangrene [20]. It has been also reported treatment with bosentan (endothelin receptor antagonists) could improve healing of the ulcers [21]. Calcium-channel blockers like nifedipine can increase distal blood flow due to peripheral vasodilation and improve circulation to the distal ischemic limb. It also can be beneficial in combination with antibiotics and iloprost [2, 22].

Surgical procedures:

Surgical modalities offer limited efficacy for the treatment of TAO. The absence of distal vascular targets makes surgical revascularization challenging. Endovascular treatment modalities have demonstrated utility lessening the progression of TAO [23]. In the setting of multi-level occlusion the stent puncture technique can help overcome vascular access challenges. [24]. Further, sympathectomy may be useful in relieving pain and promoting the

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healing of ulcers in some patients; however, these effects were not consistent [25]. Spinal cord stimulation may also be used for relief or treat pain in these patients [26].

Gene therapy

In 1998, a benchmark publication demonstrated that vascular endothelial growth factor (VEGF) gene transfer shows utility in the treatment of TAO. Isner et al. revealed the feasibility of intramuscular gene transfer of naked plasmid DNA encoding - VEGF165 in six patients affected by TAO. Following this gene transfer, patients began to show marked improvement in healing of ischemic ulcers associated with increased blood flow in affected limbs. Patients also showed improvement in ankle-brachial index (ABI) in addition to demonstrating new vessel growth with magnetic resonance angiography (MRA) and serial contrast angiography [13]. Furthermore, phase I clinical trial data demonstrated the safety of intramuscular injections of plasmid DNA expressing two isoforms of hepatocyte growth factor (HGF) (VM202) for patients with critical limb ischemia (CLI). Following plasmid DNA injection, the median ABI and transcutaneous oxygen pressure (tcPO2) values showed a positive shift, and patients responded clinically with a reduction in reported pain profile [27]. To demonstrate clinical efficacy of intramuscular plasmid injections, Belch and colleagues have evaluated intramuscular injections of non-viral 1 (NV1) fibroblast growth factor (FGF) in a phase 3 clinical trial in CLI patients. In this study, patients who were not considered suitable for revascularization were randomized to treatment with NV1FGF (naked DNA plasmid with gene encoding FGF1) or placebo. In a one-year follow-up study, the NV1FGF treated group didn't show a significant improvement in major amputation rates and mortality over the placebo group [28]. While advancements in gene therapy show hope for future therapeutic options, stem cell therapy may also play an important role in improving the quality of life in patients affected by TAO.

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Stem cell-based approaches

Clinical trials have evaluated the potential benefits of stem cell therapy in CLI [29, 30]. Reported benefits include more rapid angiogenesis, reduced inflammation, increased temperature and perfusion of the ischemic limb, and overall increased healing rates as observed by the size of the wound. Clinically, patients have lower rates of surgical amputation and report lower rates of claudication. These treatment approaches are based on the stem cells' ability to stimulate immunomodulation [8, 31] and formation of new blood vessel formation (angiogenesis) and vessel growth (vasculogenesis) [15, 32, 33]. It has been recently reported cell therapy using mononuclear stem cells (MNCs), endothelial progenitor cells (EPCs), mesenchymal stem cells (MSCs), and pluripotent stem cells (PSCs) may have useful roles in prevention of the progression of disease and reduction in major amputation rates [15, 32].

Mononuclear Stem Cells (MNCs):

MNCs isolated from bone marrow (BM-MNCs) and peripheral blood (PB-MNCs) have widely been used for cardiovascular disease applications [34]. Both BM-MNCs and PB-MNCs consists of heterogenous populations of hematopoietic stem cells, MSCs, and EPCs and their cellular composition vary depending on the purification procedure. The MNC-based cell therapy is popular because of their ease in harvesting and the implantation can be done in short turnaround time [35].

Research studies on bone marrow mononuclear cells (BM-MNCs) implantation have shown that cell therapy could increase tissue angiogenesis, neovascularization, and collateral vessel formation in both experimental models and clinical trials. **(Table 1)** The first clinical therapeutic effect of cell transplantation in angiogenesis process was shown by Tateishi-

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Yuyama et al. who evaluated therapeutic angiogenesis for patients with severe peripheral vascular disease and limb ischemia by autologous implantation of BM-MNCs. Their results revealed that use of BM-MNCs is a safe and effective method to achieve therapeutic angiogenesis [36]. In a study by Idei et al., long term clinical outcomes of BMMNC transplantation was assayed in patients with CLI including peripheral arterial disease (PAD) and TAO. Reduction of long-term major amputation risk was observed in patients with PAD who were treated with autologous BM-MNCs. In TAO patients, ABI and TcPO2 were markedly enhanced at 1 month after cell therapy and these effects remain at a high level during the 3-year follow-up [37].

Moriya et al. have shown that treatment with PB-MNCs improves ischemic symptoms and amputation rates in TAO [38]. It has been demonstrated that some factors such as granulocyte colony stimulating factor (G-CSF), granulocyte/macrophage colony stimulating factor (GM-CSF), VEGF, and estrogen induce EPCs mobilization from BM into PB, and the mobilized EPCs then localize to the neovascularization site and contribute to the repair of damaged vessels [39]. In a phase I/IIa clinical trial, cell therapy outcome was evaluated in patients with PAD or TAO. It has been reported that following intramuscular injection of PB-MNCs containing G-CSF-mobilized CD34+ stem cells, ischemic signs and symptoms involving Wong-Baker FACES pain rating scale (WBS), toe brachial pressure index (TBPI), TcPO2, total or pain-free walking distance, and size of ulcer improved in all patients. Furthermore, no mortality or major amputations were observed in this clinical study [40].

Endothelial Progenitor Cells (EPCs):

The stimulation of angiogenesis is critical to reversing the pathogenesis of TAO. Several factors are involved in the angiogenic process. It has been shown that growth factors,

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angiogenic genes, and stem cells including EPCs, are involved in modulation of angiogenesis [41]. It has been hypothesized that stem cell therapy for ischemic limbs could promote vascular angiogenesis by supplying EPCs, cytokines, and angiogenic factors. EPCs refer to the cell population that carries the ability for differentiation into endothelial cells. $CD34^+$ or $CD133^+$ ($AC133^+$) MNCs enriched EPCs can be derived from adult bone marrow (BM) or peripheral blood (PB) [42]. Human cord blood– derived CD133 progenitors implanted into the ischemic hind limbs of mice are capable of being incorporated into the capillary networks and improved neovascularization and vessel perfusion [43].

Mesenchymal Stem Cells (MSCs):

MSCs are multipotent stem cells and can be derived from various sources such as bone marrow, liver, adipose tissue, blood, and liver [44, 45]. As a multipotent stem cell, MSCs can be predominantly differentiated into cells of musculoskeletal and fat lineages and also to endothelial lineage (ref). Besides differentiating into cells of the endothelial lineage, MSCs have further contributed to immunomodulatory and angiogenic response by secreting paracrine factors and cytokines such as VEGF, basic fibroblast growth factor (bFGF), and platelet derived growth factor (PDGF), interleukin-10 (IL-10) [31, 45]. Investigations have been performed examining the potential of MSCs derived from bone marrow (BM-MSCs), umbilical cord blood (UC-MSCs), and adipose tissue (ADSCs).

BM-MSCs have widely been used to treat wounds and/or ischemic tissue due to their immunomodulatory and angiogenic effect [31, 34, 45]. In a current study Martin-Rufino et al., investigated the efficacy of sequential intravenous allogeneic MSC administration in treating TAO instead of usual local intramuscular injections [8]. The idea was to illicit a systemic anti-inflammatory effect in the vasculature and thus to modulate the immune

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response. In this single patient clinical study, the patient with TAO and at a risk of amputation was treated with four sequential intravenous infusions of allogeneic BM-MSCs, a total of 3.4 x 10^8 cells, from a healthy donor. The infusion of BM-MSCs passed the safety issue and showed no allograft rejection. Six months after the infusions the result showed significantly healed foot ulcer accompanied with reduced rest pain. Furthermore, the infusion resulted in the improvement of Walking Impairment Questionnaire scores and quality of life and the patient did not need any amputation sixteen months after the infusion. The success of this therapy can be attributed to the immunomodulatory activity BM-MSCs against IL-33 mediated inflammation in TAO [8, 11].

Studies have revealed that paracrine factors secreted by UC-MSCs stimulate angiogenesis. A preclinical study has shown that UCMSCs therapy is better than BMMSCs transplantation in improving angiogenesis in an ischemic limb disease (ILD) mouse model. UCMSCs cells can secrete high levels of HGF and upon stimulation with TNFα also produce higher amounts of VEGF compared to the BMMSCs, which are key elements for angiogenesis process during ischemia [46]. Kim et al have also demonstrated UCMSCs transplantation can produce effective outcomes in TAO. Cell therapy improves ischemic symptoms including alleviation of ischemic rest pain, healing of necrotic skin lesions associated with an increase in the size and density of capillaries [47].

Studies have been recently reported that adipose tissue derived MSCs (ADSCs) can be differentiated into endothelial cells and enhance micro-vascularity and blood flow in CLI animal models. The mechanism by which ADSCs induce therapeutic angiogenesis is through the secretion of angiogenic factors such as VEGF [48-51]. Lee et al. determined the safety and efficacy of multiple intramuscular transplantations of ADSCs are safe and effective

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demonstrating improved pain rating scales and pain-free walking distances and limb amputation rates. The collateral vascular network formation was also detected in the affected arteries using angiography [32][52].

Pluripotent Stem Cells (PSCs):

Pluripotent stem cells have the ability to self-renew infinitely and can be any cells of the body. These PSCs can be derived either from inner cell mass of the blastocyst to become embryonic stem cells (ESCs), or from somatic cells by expressing specific transcription factors (e.g. Oct4, Sox-2, Klf-4 and c-myc) to form induced pluripotent stem cells (iPSCs) [53, 54]. The use of iPSCs are advantageous as they avoid ethical concerns associated with ESCs. Both ESCs and iPSCs have already been used to derive vascular cells such as endothelial (EC) and vascular smooth muscle cells (VSMC) to induce neovascularization. In two separate studies Cho et al., [55] and Huang et al. [56] demonstrated efficacy of ESC-derived endothelial cells in inducing neovascularization in animal model of CLI. While intramuscular (IM) injection of ESC-ECs by Cho et al., resulted in improved blood perfusion and limb salvage, the study by Huang et el., compared various routes of cell delivery and found systemic route to be more efficient in improving blood perfusion and neovascularization than IM [55, 56]. In another study, Yamahara et al., used a combination of ESC-derived ECs and VSMCs to treat ischemic limb [57]. The result showed formation of mature vasculature compared to EC or SMC alone. These initial studies have led to the generation of clinical grade ESC-EC product developed using good manufacturing practice to use them to reperfuse ischemic limb in patients [58].

Similar to ESCs, iPSCs have been used to derive functional ECs. An initial study by Rufaihah et al. showed the efficacy of these cells in improving blood perfusion and

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neovascularization in an animal model of limb ischemia [59]. In order to further establish PSCs as a functional source of ECs, Lai et al., compared ECs derived from various sources including bone marrow (BM-EC), ESCs, and iPSCs in their ability to improve neovascularization and secretion of paracrine factors [60]. The ESC- and iPSC-EC outperformed BM-EC in their ability to secrete paracrine factors and have similar in vivo efficiency in inducing neovascularization as compared to ECs derived from human umbilical vein. These data further support the feasibility of using human PSC-ECs in developing novel cell therapies for patients with CLI and TAO.

Conclusion

In the past two decades, stem cell-based therapy has demonstrated clinical efficacy in the form of therapeutic angiogenesis in peripheral vascular disease. Use of cell therapy for induction of angiogenesis as well as immunomodulation in the animal model has slowly progressed into human clinical trials. While the angiogenic and immunomodulatory potentials of stem cell-based therapy have been demonstrated in human clinical trials of both CLI and TAO, there are still many challenges. The factors that would determine the success of these cell therapies in near future are i) understanding the mechanism and therapeutic potentials of cell-based therapy; ii) establishing a large-scale and renewable source of autologous cells; iii) developing strategies to improve cell potency in vivo and iv) deciding optimal dose, efficient route of administration and frequency of application. Ultimately, we will also need to understand how the in vivo tissue microenvironmental affect the therapeutic activity.

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Study	Year	Patients (n)	Cell Source	Cell Route	Follow up	Main Results	Ref.
Kim, D.I et al.	2006	27	BMMSCs	fenestration of the tibia bone	19.1 ± 3.5 months (range, 12.4 to ± 25 months)	inducing therapeutic angiogenesis	[9]
Heo et al.	2016	37	BMMSCs	IM	11.9 ± 7.2 months	Improvement of TBPI and healing of ischemic wounds, pain relief	[23]
Idei et al.	2011	26	BMMSCs	IM	4.8 years	Increase in ABI, $TcPO_2$ In 3years follow up	[37]
Kim, S.W et al.	2006	4	UCBMSCs	IM	1&4 Months	Disappearance of Ischemic rest pain, healing of necrotic skin lesions, Increase in number and size of capillaries	[36]
Lee et al.	2012	15 male CLI patients Including TAO	ADSCs	IM	6 months	therapeutic angiogenesis	[24]
Ra et al.	2017	17	ADSCs	IM	2 year	increase in TWD, PFWD And decrease in rest pain.	[41]

Table 1: Stem cell therapy clinical studies in TAO patients

BMMSC, bone marrow mesenchymal stem cell; UCMSCs, umbilical cord blood derived mesenchymal stem cells; ADSCs, adipose tissue derived mesenchymal stem cells; TBPI, toe brachial pressure index ; $TcPO_2$, transcutaneous partial oxygen pressure; ABI, ankle-brachial index. TWD, total walking distance; PFWD, pain free walking distance.IM, Intramuscular.

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