Hope for Therapeutic Angiogenesis for Patients with Critical Limb Ischemia: from Bench to Bedside

Chao-Hung Wang,[1] Subodh Verma[2] and Wen-Jin Cherng[1]

Peripheral arterial disease is a common but under-recognized problem. As the disease progresses, the patient might suffer from rest pain and/or ischemic ulceration — critical limb ischemia (CLI). The primary goals for treating the limb symptoms of CLI are to improve functional capacity, exercise performance and qualify of life. Although numerous therapy options can be used for CLI, unfortunately, about 20-30% of patients with CLI cannot be treated successfully and the only option for them is often amputation. For this group of patients, there is a great need for alternative treatment strategies, and several strategies to stimulate new vessel formation are currently being tested. These include gene therapy, angiogenic cytokine therapy, and cell therapy (such as with bone marrow-derived mononuclear cells and endothelial progenitor cells). In preclinical studies, most of these therapies have promoted the development of collateral arteries and demonstrated beneficial effects in patients with CLI. However, a great deal of more clinical experience is needed to resolve safety concerns such as potentiation of pathological angiogenesis (e.g., malignancy) and so-called bystander effects of delivered factors (e.g., effects on kidney or atheroma). These concerns limit their clinical applications at the present time. Currently, extensive clinical trials on angiogenesis are underway in Japan and worldwide. However, most of the studies are neither placebo-controlled nor randomized. The focus of future research on collateral artery growth should shift back from exaggerated enthusiasm to clear, hypothesis-driven studies, hopefully providing better insights into the exact molecular mechanisms of postnatal vessel growth. Despite these hurdles on the way to clinical application, the stimulation of collateral artery growth undoubtedly remains one of the most exciting challenges facing the medical scientific community. Given the large number of patients that will benefit from such therapy, accomplishing this task would be a revolutionary step in cardiovascular medicine.

Key Words: Critical limb ischemia • Gene therapy • Growth factor therapy • Cell therapy • Stem cell therapy

CLINICAL PROBLEMS AND AVAILABLE TREATMENT

Peripheral arterial disease (PAD) is a common but under-recognized problem. Intermittent claudication is the most frequent symptom of PAD, although the diagnosis of PAD is often overlooked until the patient presents with limb-threatening ischemia. As the disease progresses, the patient might suffer from rest pain and/or ischemic ulceration — critical limb ischemia (CLI). Critical limb ischemia implies chronicity and is to be distinguished from acute limb ischemia. Its incidence is approximately 500 to 1000 per million inhabitants, with the highest rates among older subjects, smokers and diabetics.[1]

The primary goals for treating the limb symptoms of PAD are to improve functional capacity, exercise performance and qualify of life. Current therapeutic options in-

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1Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung; Chang Gung University College of Medicine, Taiwan. [2]Division of Cardiac Surgery, St. Michael's Hospital, Toronto, Canada.
Address correspondence and reprint requests to: Dr. Chao-Hung Wang, MD, Division of Cardiology, Department of Internal Medicine, Chang Gung Memorial Hospital, 222, Mai-Chin Road, Keelung, Taiwan. Tel: 886-2-2431-3131, ext. 3168; Fax. 886-2-2433-5342; bearty@adm.cgmh.org.tw

clude a variety of medical or surgical modalities as follows. Exercise training in a formal setting, revascularization with angioplasty and cilostazol all have proven efficacy.[2] Prostaglandins do not have a documented role in treating claudication. Carnitine and its derivatives (propionyl-L-carnitine) have been shown to improve treadmill exercise performance and quality of life. These drugs also have an excellent safety profile. Statins have been demonstrated to not only reduce the increased risk of ischemic events but also appear to improve claudication symptoms.[2,3] On the other hand, all patients with PAD should receive antiplatelet therapy to prevent ischemic events and angiotensin-converting enzyme inhibitors if appropriate. It is also recognized that selected patients with claudication symptoms may benefit from catheter-based interventions, and most PAD patients with critical leg ischemia require revascularization procedures. Although many therapies for claudication have been thoroughly investigated, unfortunately, about 20-30% of patients with CLI,[4] such as those with no graftable distal vessels present, or neurologically impaired or hopelessly nonambulatory patients, cannot be treated by any of the methods as mentioned above and the only option for them is often amputation.[4] For this group of patients, there is a great need for alternative treatment strategies. Several strategies to stimulate new vessel formation (i.e. arteriogenesis) are currently being tested.

EXPERIMENTAL AND ANIMAL STUDIES OF THERAPEUTIC ANGIOGENESIS

Three distinct mechanisms of new vessel formation have been identified: vascuologenesis, angiogenesis, and arteriogenesis. Numerous animal studies demonstrated the potential of a variety of experimental therapies in angiogenesis and vasculogenesis for ischemic diseases. These include gene therapy, angiogenic cytokine therapy, and cell therapy.

Growth factors and gene therapy: experimental evidence

A variety of genes and growth factors stimulate vascular cell proliferation and maturation with following angiogenesis.[5] Some of them may enhance tissue regeneration not solely via the proangiogenic activity but also via promotion of stem/progenitor cell mobilization.[6,7] Potential therapeutic genes or growth factors for improvement of angiogenesis or arteriogenesis include the target on vascular endothelial growth factor (VEGF, mobilization and differentiation of EPCs, and stimulation of endothelial cell proliferation),[8] placenta-derived growth factor (PlGF, stimulation of ischemic tissue revascularization),[9] fibroblast growth factor (FGF-stimulating growth of EPCs and smooth muscle cells),[10] angiopoietin-1 (mobilization of EPCs and other progenitor cells),[11] hepatocyte growth factor (HGF, stimulation of endothelial cells and attraction of tissue-resident cardiac stem cells),[12] insulin-like growth factor (IGF-stimulating the growth of endothelial and smooth muscle cells, and progenitor cells),[13] erythropoietin (mobilization of EPCs),[11] and granulocyte monocyte-colony stimulating factor (GM-CSF, mobilization of stem cells and EPCs).[11]

Results of preclinical studies have shown that angiogenic growth factors or genes encoding these proteins promote the development of collateral arteries, which is called therapeutic angiogenesis.[14] Limited clinical data from protein-delivery and gene-delivery trials suggest that both approaches are safe.[15,16] In view of the enclosure of formed mature vessels with periendothelial matrix and pericytes, smooth-muscle cells, or both, treatment with various angiogenic growth factors might be preferable in future treatments.[17] However, a great deal more clinical experience is needed to resolve safety concerns such as potentiation of pathological angiogenesis (e.g., malignancy) and so-called bystander effects of delivered factors (e.g., effects on kidney or atheroma).[18]

Cell therapy for therapeutic vascularization

Bone marrow-derived mononuclear cells

Previous reports[19,20] noted that bone marrow-derived mononuclear cells from adult humans improved capillary density in hindlimb ischemia. Bone marrow-derived mononuclear cell implantation into ischemic limbs[21] promotes collateral vessel formation, with incorporation of endothelial progenitor cells into new capillaries.

Matsubara and colleagues reported that a significant portion of the bone marrow-derived mononuclear cells synthesized not only angiogenic growth factors (VEGF and basic FGF) but also angiopoietin-1, interleukin 1β and tumour necrosis factor α, which are known to have important functions in maturation and maintenance of

the vascular system.[22] Takakura and colleagues also reported that marrow hematopoietic cells release angiopoietin-1 to induce the maturation of endothelial progenitor cells.[23] The multiple cytokines released by these bone marrow-derived mononuclear cells may also stimulate adequate enclosure of vessels by periendothelial matrix and pericytes, and smooth-muscle cells. These phenomena significantly improve the formation of stable capillary vessels.

Before extensively applying this therapeutic modality to human trials, one concern is that marrow cells could differentiate into various mesenchymal cells, since marrow cells include cells of various lineages, such as fibroblasts, osteoblasts, myogenic cells, and endothelial cells.[24] However, animal studies showed that injected bone-marrow mononuclear cells are unlikely to have the ability to differentiate into fibroblasts, osteoblasts, and myogenic cells in ischemic tissues.[21,25]

Endothelial progenitor cells and postnatal vasculogenesis: experimental evidence

Endothelial progenitor cells (EPCs) have recently been identified from adult species and shown to possess therapeutic potential in a variety of diseases caused by atherosclerosis.[26,27] EPCs are mobilized from the bone marrow into the circulation, home to the site of vessel injury in response to physiological and pathological stimuli, and then differentiate into endothelial lineage cells, thus contributing to postnatal neovascularization.[28] To date, EPCs have been applied in tissue engineering for improving the biocompatibility of vascular grafts,[29,30] have been used to promote neovascularization of the adult brain following stroke[31] and of ischemic limbs,[32,33] and have been demonstrated to preserve left ventricular function following myocardial ischemic injury.[34] The marker of angioblasts and hematopoietic stem cells, CD34, is used to isolate putative angioblasts from the leukocyte fraction of peripheral blood.[35] Endothelial progenitor cells can also be isolated from human umbilical cord blood,[36] bone marrow-derived mononuclear cells,[37] and $CD34^+$ or $CD133^+$ hematopoietic stem cells.[35,38] Importantly, EPCs can be successfully ex vivo expanded with the use of human peripheral blood mononuclear cells.[39] Some of the EPCs were found to be able to differentiate into so-called late-outgrowth endothelial cells with expression of a panel of endothelial cell markers and uptake of Dil-acetylated LDL and binding of lectin.[35,40-41] In animal models of ischemia, endothelial progenitor cells have been shown to incorporate into the formation of neovascularization in ischemic tissue with markedly improved blood flow recovery and capillary density and a reduced rate of limb loss.[33-34,42-44] EPC therapy is also able to increase impaired blood flow in diabetic mice.[45] These findings provide evidence that exogenously administered endothelial progenitor cells augment naturally impaired neovascularization in animal models of experimentally induced ischemia.

HUMAN CLINICAL TRIALS

Therapeutic vessel growth for critical ischemia: evidence from human trials

Growth factors and gene therapy

In "no-option" patients with disabling ischemia despite all possibilities for medical or surgical revascularization therapies, the development of biological revascularization has been intentionally attempted with the use of recombinant growth factor proteins or gene therapy.

The goal of therapeutic angiogenesis is to stimulate new blood vessel growth. Although there have not been many studies in peripheral artery disease, a few large randomized placebo-controlled trials have been performed in the adult heart to improve myocardial perfusion and function. The results of these trials can probably be applied to the therapy in patients with peripheral artery occlusion disease (Table 1). The FIRST study (FGF-2 Initiating Revascularization Support Trial) used basic FGF protein versus placebo.[46] In the FIRST trial, 337 patients were randomized to a single intracoronary infusion of rFGF-2 at 0.3, 3, or 30 ug/kg or placebo. This study failed to demonstrate significant improvement at the endpoints. Post hoc analysis suggested a significant improvement in exercise time only in patients over 65 years of age with severe ischemia symptoms. The VIVA trial (VEGF in Ischemia for Vascular Angiogenesis) used VEGF-1 protein.[47] Totally, 178 patients with stable exertional angina, unsuitable for standard revascularization, were randomized to placebo, low-dose or high-dose rhVEGF165 delivered via intracoronary infusion on day 0, followed by intravenous infusion on days 3, 6, and 9. At day 120, there was a significant improvement in an-

Table 1. Phase II clinical protein and gene therapy trials in patients with ischemic heart disease

Trial name	Protein or Vector	Route of Administration	No. of patients	Follow-up	Results		References
					Primary endpoints	Secondary endpoints	
Protein therapy							
FIRST	FGF-2	Intracoronary	337	90d to 6 mo	Negative (ET)	Negative (MP, ABI)	44
VIVA	$VEGF_{165}$	Intra-myocardial	178	60, 120d, 1y	Negative (ETT)	Negative (AC, high dose positive at 4 months (AC))	45
Gene therapy							
AGENT II	FGF-4 (adenovirus)	Intracoronary	79	4 and 12 wk	Negative	Subgroup analysis positive (IDS, RPDS)	16
REVASC	$VEGF_{121}$ (adenovirus)	Intra-myocardial (surgery)	67	12, 26 weeks	Not defined	Positive (TET at 26 weeks)	46

AC, angina class; ABI, ankle-brachial index; ET, exercise tolerance; ETT, exercise treadmill test; FGF, fibroblast growth factor; MP, myocardial perfusion; IDS, ischemic defect size; RPDS, reversible perfusion defect size; TET, time to ST depression on exercise tolerance; VEGF, vascular endothelial growth factor.

gina class. However, all other day 120 endpoints were not statistically different. The AGENT (Angiogenic GENe Therapy) trials tried replication-defective adenovirus containing the FGF4 gene (Ad5-FGF4).[16] AGENT I and II showed that in the subset of patients with severe (class III or IV) angina, there were trends toward improvement. The trials reported that the Ad5FGF-4 treatment was well tolerated and did not result in any permanent adverse sequelae; however, primary endpoints of efficiency were not reached. The REVASC study (Randomized Evaluation of VEGF for Angiogenesis in Severe Coronary disease), a phase II trial, is the first large randomized gene therapy trial,[48] using replication-defective adenovirus containing the VEGF121 gene (AdVEGF121). The adenoviral-based vector was injected intramyocardially during surgery to 32 nooption patients. At 6-week follow-up, angina class dropped significantly in the treated group. However, 2 patients died due to postoperative complications in the treatment group.

Regarding peripheral artery occlusion disease, the TRAFFIC trial (TheRapeutic Angiogenesis with recombinant Fibroblast growth Factor-2 for Intermittent Claudication trial) enrolled 190 patients with severe peripheral arterial disease and claudication (Table 2).[49] The rFGF-2 was delivered via intra-arterial infusion to the lower extremities bilaterally. Intention-to-treat analysis demonstrated significant improvemt in the rFGF-2 arms at 90 days. However, by day 180, there was no difference between groups. The Regional Angiogenesis with Vascular Endothelial growth factor (RAVE) trial was the first major randomized study of adenoviral VEGF gene transfer for the treatment of peripheral artery disease.[15] This phase II, double-blind, placebo-controlled study was designed to test the efficacy and safety of intramuscular delivery of AdVEGF121, a replication-deficient adenovirus encoding the 121-amino-acid isoform of VEGF, to lower extremities of subjects with unilateral PAD. RAVE investigators concluded that a single intramuscular administration of AdVEGF121 was not associated with improved exercise performance or quality of life. Their study did not support local delivery of single-dose VEGF121 as a treatment strategy in patients with unilateral PAD.

In summary, these results suggest that a "single-shot" design with protein is not sufficient for therapeutic angiogenesis, since the half-life of protein is too short. On the other hand, most of the reports also suggested that single gene transfer for the majority of patients was ineffective.

Although current efforts are focused on targeting angiogenesis to ischemic tissues, there exists the theoretical risk of unwanted blood vessel growth in adjacent or distant tissue sites.[18] VEGF is known to increase vascular permeability and tissue edema and to cause hypotension, while FGF therapy is associated with proteinuria. Broader safety concerns include the possibility of accelerating occult tumor growth, diabetic retinopathy, or atherosclerosis. These concerns limit their clinical applications at present.

Table 2. Clinical protein and gene therapy trials in patients with peripheral artery occlusive disease

Trial name	Protein or Vector	Delivery method	No. of patients (disease)	Follow-up	Results		References
					Primary endpoints	Secondary endpoints	
Protein therapy							
TRAFFIC	rFGF-2	Intra-arterial	174	3 months	Negative*	Negative	47
Hisayoshi et al.	G-CSF	Intra-venous	N.A.	Ongoing			
Masazumi et al.	G-CSF	Intra-venous	N.A.	Ongoing			
Yoshikazu et al.	bFGF/FGF-2	Intra-muscular	N.A.	Ongoing			
Gene therapy							
RAVE	$VEGF_{121}$ (adenovirus)	Intra-muscular	105	12, 26 months	Negative (ET, QOL)	N.A.	15
TREAT-HGF	HGF	Intra-muscular	N.A.	Ongoing			
Cell therapy							
TACT	BMC	Intra-muscular	45	3 months	Positive (ET, QOL)	N.A.	22
Kudo	EPC	Intra-muscular	2	2 weeks	Positive ($TcPO_2$)	N.A.	51
Atsuhiko et al.	EPC	Intra-muscular	N.A.	Ongoing			

BMC, bone marrow cell; bFGF, basic fibroblast growth factor; ET, exercise tolerance; G-CSF, granulocyte-colony stimulating factor; HGF, hepatic growth factor; $TcPO_2$, transcutaneous oxygen pressure; VEGF, vascular endothelial growth factor; QOL, quality of life. *, positive after evaluating 190 patients.

Cell therapy for critical ischemia: evidence from human trials

The intriguing results from experimental studies of gene and protein therapy as mentioned above promoted the initiation of clinical cell therapy pilot trials. In 2002, Tateishi-Yuyama et al.[22] and the Therapeutic Angiogenesis by Cell Transplantation (TACT) study investigators performed a randomized controlled trial in patients with PAD. These investigators found that, after intramuscular injection of bone marrow-derived mononuclear cells, a significant increase in transcutaneous oxygen pressure, rest pain, and pain-free walking time was noted in most of the patients with leg ischemia.

The success of bone marrow-derived mononuclear cells in the treatment of critical limb ischemia is well explained as follows. Bone marrow-derived mononuclear cells contain both $CD34^+$ and $CD34^-$ fractions. Endothelial progenitor cells in the $CD34^+$ stem-cell fraction take part in postnatal neovascularisation.[35,50] Infusion of endothelial progenitor cells has been shown to induce angiogenesis in ischemic limbs,[19,20] This line of evidence can be supported by the findings that peripheral blood-derived mononuclear cells devoid of endothelial progenitor cells had weaker angiogenic activity. Knowledge gained from studies in the past few years suggests that efficacy of implantation of bone marrow-derived mononuclear cells is due to supply of endothelial progenitor cells (included in $CD34^+$ fraction) and multiple angiogenic factors (released from $CD34^-$ fraction). The $CD34^-$ fraction in bone marrow-derived mononuclear cells synthesized not only angiogenic growth factors (VEGF and basic FGF) but also angiopoietin-1, which is known to have important functions in maturation and maintenance of the vascular system.[23,51] When considering clinical potential of therapeutic angiogenesis, for newly formed vessels to survive, they must be remodelled and acquire a smooth-muscle coat with adequate enclosure of vessels by periendothelial matrix and pericytes, and smooth-muscle cells.[52] These combined effects derived from bone marrow-mononuclear cells, mixing $CD34^+$ and $CD34^-$, could lead to the formation of stable capillary vessels.

As mentioned regarding the animal studies, marrow cells include cells of various lineages. Such mixed popu-

lations could differentiate into various mesenchymal cells.[24] In the TACT study, investigators immunohistochemically investigated marrow-implanted limbs. Apparent increases in capillary numbers were noted, whereas neither bone formation nor increased interstitial fibrosis was detected. Thus, in ischemic limbs, endothelial-lineage cells could effectively differentiate into mature cells, whereas some survival factors to stabilise other marrow-derived lineage cells could be lacking in ischemic conditions. On the other hand, the TACT study demonstrated that marrow implantation did not affect circulating concentrations of angiogenic growth factors. Combinations of these growth factors locally secreted from the marrow cells might be useful in further treatments directed toward neovascularisation of tissues without adverse effect on retinopathy or the concerns on tumor growth.

Regarding use of the circulating endothelial progenitor cells from peripheral blood as a more convenient source, Kudo and colleagues have been investigating the use of autologous transplantation of peripheral blood endothelial progenitor cells for therapeutic angiogenesis in patients with critical limb ischemia.[53] However, to achieve a functional improvement, endothelial progenitor cells needed to be ex vivo cultured to enrich an active subpopulation (which is maximally approximately 0.5% of the total mononuclear cells) out of the peripheral blood mononuclear cells. Otherwise, freshly isolated EPC-containing peripheral blood monocytes did not exert any effect. This process raises the safety concern over the implantation of ex vivo cultured cells back to ischemic tissues and hampers its clinical applications at the current stage.

Some investigators have been trying to increase the mobilization of endothelial progenitor cells to circulation before harvesting from peripheral blood. Pretreatment with G-CSF can increase endothelial progenitor cells in bone marrow or peripheral blood, which could reduce the aspiration volume of marrow cells required or enhance efficacy of collateral vessel formation after implantation of bone marrow-mononuclear cells. In two reports, injection of GM-CSF was noted to mobilise endothelial progenitor cells from bone marrow[54] or promote cardiac collateral growth in patients with coronary artery disease.[55] However, angina pectoris or acute[56] arterial thrombosis[57] arise in patients receiving G-CSF (granulocyte-colony stimulating factor) because of leucocytosis or hypercoagulability. Since most patients eligible for cell therapy are predicted to have severe atherosclerotic lesions in coronary or cerebral arteries, pretreatment with G-CSF might cause deleterious vascular events before angiogenic cell therapy. On the basis of these findings, clinical trial adopting pretreatment with these factors raises safety concerns.

Although increased perfusion was demonstrated in most of the studies as described above, these studies at present are limited by the small patient samples and by the design of pilot safety and feasibility studies.[58-61] The TACT study[25] is the first to show the efficacy and safety of implantation of bone marrow-derived mononuclear cells in 45 ischemic limbs. Legs that were injected with peripheral blood-derived mononuclear cells showed much smaller increases in collateral perfusion, suggesting in favor of bone marrow-derived mononuclear cells as an effective therapeutic strategy. Furthermore, autologous implantation of bone marrow-mononuclear cells can also constitute a safe strategy for achievement of therapeutic angiogenesis without cell rejection.

CURRENT STATUS OF THERAPEUTIC TRIALS IN A CLINICAL SETTING

Currently, in addition to the bone marrow implantation strategies mentioned above, extensive clinical trials on angiogenesis are ongoing in Japan and worldwide (Table 2). These clinical trials include: (1) Endothelial progenitor cells in Angiogenesis: Dr. Kawamoto Atsuhiko (Japan); Dr. Asahara (Japan); (2) Hepatocyte growth factor in angiogenesis — TREAT-HGF (Japan trial to Treat Peripheral Arterial Disease by Therapeutic Angiogenesis Using Hepatocyte Growth Factor Gene Transfer); (3) G-CSF in Angiogenesis: Dr. Fujiwara Hisayoshi (Japan); Dr. Arai Masazumi (Japan); (4) bFGF/FGF-2 trials in Angiogenesis: Dr. Yonemitsu Yoshikazu (Japan). However, all these studies are right in the middle of their courses and not finished. Unpublished data has shown promising effects based on these treatment modalities, however, safety results have not been released yet (The 37th Japan Atherosclerosis Society Annual Meeting. WellVAS 2005;11).

The TACT study proved that autologous implanation

of bone marrow-derived mononuclear cells could be safe and effective for achievement of therapeutic angiogenesis in patients with ischemic limbs because of PAD. After this report was published, Dr. Matsubara organized more than 20 medical centers in Japan to undertake an extensive clinical trial based on the same therapeutic strategy. Unpublished data showed that this strategy generally caused a 65% improve in ankle-brachial index, an 80% improve in rest pain, 3 minutes longer pain-free walking time, and, most importantly, a 75% decrease in amputation rate and a 60% improve in limb ulceration (The 37th Japan Atherosclerosis Society Annual Meeting. WellVAS 2005;11). Additionally, with regard to adverse effects associated with this intervention, no infection, inflammation or increases of any specific systemic cytokines have been noted or reported. The procedure of bone marrow aspiration is just a regular procedure for hematologists doing bone marrow transplantation and can be safely performed by experienced hands without safety issues. The only supportive treatment is for those patients with Hb < 10 gm/dl, who need blood transfusion after bone marrow aspiration. Basically, this therapeutic procedure is perfectly acceptable on safety grounds.

Given the findings that transplantation with whole bone marrow cells holds promise in the efficacy of cell therapy for critical limb ischemia, there may be a future attempt to mix a few different populations of potential cells for cell transplantation. Recently, Yoon et al. cultured different types of endothelial progenitor cells from peripheral mononuclear cells.[62] These different populations produced beneficial profiles of cytokines and proteases with synergistic impacts on neovascularization. The transplantation of mixed types of cells resulted in synergistic augmentation of angiogenesis. Rafii and Lyden et al. also supported that such synergistic interactions may be present among a variety of stem or progenitor cells, possibly shedding light on a future direction of stem cell therapy.[63]

However, as mentioned above, most of the early trials evaluating the stimulation of vascular growth were neither placebo-controlled nor randomized. The focus of future research on collateral artery growth should shift back from exaggerated enthusiasm to clear, hypothesis-driven studies, hopefully providing better insights into the exact molecular mechanisms of postnatal vessel growth. Actually, only class IIb indication is granted for angiogenic growth factor therapy for peripheral artery disease in the recent 2006 AHA guidelines.[3] Despite these hurdles on the way to clinical application, the stimulation of collateral artery growth undoubtedly remains one of the most exciting challenges facing the medical scientific community. Given the large number of patients that will benefit from such therapy, accomplishing this task would be a revolutionary step in cardiovascular medicine.

QUALITY OF PRACTICE AND CONCERNS OF COST AND BENEFIT

More and more new strategies for the treatment of peripheral artery occlusive disease are being designed. Cell therapy holds great promise for improving peripheral ischemia in the future. However, when new cell therapies are being developed, it must be kept in mind that we have to perform all the possible treatments in line with the guidelines of good laboratory practice (GLP), good clinical practice (GCP), and good tissue preparation. GLP briefly contains two parts, namely, the hardware and the software. The hardware includes tissue or cell harvesting, isolation, preservation and delivery systems. The software means the responsibility to manage the research database, including projects, papers, awards and patents, under the guidelines of GCP at the same time.

The costs and benefits, and the advantages and disadvantages, of an alternative therapy should be considered when adopting a new treatment strategy for a patient. For example, the procedures of cell therapy with whole BMCs include BM aspiration, general anesthesia, cell separation and cell injection. Generally, it costs around NT$ 22,000 per patient. The disadvantages are that the patient has to take the risk of general anesthesia and BM aspiration, and that the expenditure is higher than that for an angioplasty procedure (approximately NT$ 10800-13000 per angioplasty). However, these new angiogenesis strategies, of course, have benefits in a “non-option” patient before a limb amputation is performed.

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嚴重肢端缺氧病人治療之新希望

王兆弘[1] Subodh Verma[2] 程文俊[1]
基隆市 財團法人長庚紀念醫院基隆分院 心臟內科[1]
Division of Cardiac Surgery, St. Michael's Hospital, Toronto, Canada[2]

週邊動脈血管疾病是一個常見但往往不容易被發現的問題，隨著疾病的進展，這些病人可能會有休息態疼痛或者是缺氧性潰瘍的發生，我們稱之為嚴重肢體缺氧。治療這類病人的基本目的就是要改善他們的功能狀態、運動能力以及生活品質。雖然目前對於這種嚴重肢端缺氧的病人有相當多的治療方法，但是不幸的仍有 20%-30% 的病人治療無效而必須接受截肢手術。對於這一類的病人有極大的需要去發展新的治療方法來刺激新血管的形成以改善缺氧情形。這些方法包括基因治療、成長素治療或是細胞治療，例如幹細胞治療。目前研究顯示大部分這些新的治療方法似乎都可改善缺氧狀態，但是隨著治療所帶來的一些副作用和一些意想不到的不良效果則是我們必須注意的安全考量，這些安全考量也大大的限制了新治療方法的臨床應用。目前全世界有大量的臨床試驗及研究在進行中，然而大部分這一些都不是安慰劑控制型的或是隨機型的。將來的研究設計應該更為嚴謹以提供更正確的資訊。雖在應用到臨床治療方面的困難相當多，但是基於全世界有龐大數目的病人可能會受益於這些治療，若能夠克服這些困難將是在心臟血管醫療史上革命性的進展。

關鍵詞：嚴重肢體缺氧、基因治療、成長素治療、細胞治療、幹細胞治療。