Adipose derived stem cells for regenerative cell therapy in peripheral vascular disease

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Abstract
Regenerative medicine is the emerging field that requires a reliable source of stem cells in addition to biomaterial scaffolds and cytokine growth factors. Adipose tissue has proven to serve as a source of autologous adult stem cells that can be obtained repeatedly in large quantities under local anaesthesia with a minimum of patient discomfort. Compared with bone marrow-derived mesenchymal stem cells, adipose derived stem cells (ADSC) do have an equal potential to differentiate into cells of a lineage specific required cell. Adult stem cells hold, through unclear mechanism, a great promise for repair and regenerate tissue in peripheral vascular disease (PVD). In PVD there is an obstruction in large arteries in legs and arms, what cause an ischemic area. Although some clinical experience with stem cell therapy in PVD are promising, it is to early for general use of this technique. Long-term effects of these kinds of therapies still have to be investigated, such as the progressive role of vascular endothelial growth factor in atherosclerosis and the role of ADSC in carcinogenesis. In this paper there is a representation of the use of ADSC as cell therapy in PVD patients.

Keywords Regenerative cell therapy, peripheral vascular disease, adipose derived stem cells

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1. Introduction

Peripheral vascular disease (PVD) is a common syndrome that affects a large proportion of the adult population worldwide (1). PVD is a growing medical problem in Western society, and it is expected that the prevalence will even further increase, and reach an epidemic proportion, due to a steady increase in the occurrence of associated risk factors, such as ageing, diabetes, obesity, and hypertension (2). PVD includes all diseases caused by the obstruction of large arteries in the arms and legs, causing inadequate oxygen supply to the limbs and thereby introducing an ischemic area. Usually two clinical presentations of PVD are distinguished: intermittent claudication (IC) and critical limb ischemia (CLI). IC is characterized by pain upon walking and CLI is a more severe form in which pain occurs at rest and which is accompanied by necrosis and ulceration (2). Treatment options for PVD patients are limited, and with the current therapy approximately one-fourth of the patients cannot be helped (3).

PVD is in general, but not always, a consequence of an atherosclerotic process (4). Atherosclerosis is an inflammatory disease characterized by leukocyte infiltrations, smooth muscle cell proliferation, and neointima formation (5). Activation, and damaging of the endothelial monolayer seems to trigger the development of the lesions. The endothelial monolayer, the cell layer that forms the inner lining of blood vessels, plays a crucial role in mediating homeostasis. The participation of the endothelial monolayer in theoretical every disease makes the endothelial monolayer a diagnostic and therapeutic potential. The participation of the endothelial monolayer in a disease can be as a primary determinant of pathophysiology, or as a victim of collateral damage. (6) Evidence that endothelial dysfunction is involved in the pathophysiology of PVD is, for example, coming from the finding that the administration of l-arginine, the precursor of nitric oxide, improves walking capacity in patients. Furthermore enhancing the formation and/or release of nitric oxide by improving the peripheral blood flow, for example, through physical training, statins, and ACE inhibitors, increases the exercise tolerance in PVD patients (7) (8).

Endothelial progenitor cells (EPC) may play an important part in endothelial repair and replacement of dysfunctional, or damaged endothelial monolayer. Low levels of EPC are associated with a worse cardiovascular outcome (7). It has been demonstrated that tissue ischemia and cytokines mobilize bone marrow derived EPCs to ischemic areas to form adult blood vessels (9). Therefore EPC have been implicated both in the pathogenesis of, and as a treatment for many conditions, including atherosclerosis (10). Bone marrow derived EPC, a proportion of the circulating blood EPC, have the capacity to differentiate into functional endothelial cells (EC), and participate in endothelial repair and the process of incorporation into new blood vessels in an ischemic tissue (11). Recent studies demonstrated that vascular progenitor cells recruited, and incorporated into the atherosclerotic lesion (12). Although the mechanism involved is still unclear, EPC seem to contribute to the restoration of the endothelial monolayer. In experimental models of cardiovascular ischemia allogeneic, and

autologous EPC have been shown to incorporate into sites of active neovascularisation and re-endothelialization (3).

In the course of new blood vessel formation, two different processes, vasculogenesis and angiogenesis, have to be distinguished. The term vasculogenesis describes the *de novo* emergence of a vascular network by endothelial progenitors, whereas angiogenesis corresponds to the generation of vessels by sprouting from pre-existing capillaries. Until recently, it was thought that vasculogenesis is restricted to the prenatal period. (13)

For patients that cannot be helped by the current therapies, experimental non-invasive revascularization strategies, such as angiogenic growth factor therapies, have been introduced. The benefit for PVD patients was disappointing (2). In this context the concept of stem cell-based revascularization is suggested. The general idea behind the concept of stem cell-based therapy is that EPC/functional EC repair and replace dysfunctional, or damaged endothelial monolayer.

A stem cell is, by definition, characterized by its ability to undergo self-renewal and its ability to undergo multilineage differentiation and form terminally differentiated cells (14). Postnatal (adult) stem cells are pluripotent at best and these cells retain a broad differentiation potential, although it is more restricted than embryonic stem cells (ES), which are totipotent (14). Postnatal adult stem cells reservoirs are found, for example, in the gut and the bone marrow. Moreover, all organs appear to harbour tissue specific stem cells, so tissue specific stem cells can be derived from specific organs, such as brain, gut, lung, liver, adipose tissue, and bone marrow (15). Adult stem cells hold great promise for use in tissue repair and regenerations (16). Stem cells, or progenitor cells can be activated by environmental stimuli to participate in various forms of tissue regeneration. Stem cells have in contradistinction to progenitor cells the ability to undergo self-renewal. Progenitor cells have an higher cycle number than stem cells. Besides the constructive role, the stem cells or progenitor cells, can also have an instructive role.

In the past few years, interest has rapidly grown in the developmental plasticity and therapeutic potential of stem cells isolated from adipose tissue (17). It is demonstrated that stem cells in the human adipose tissue can differentiate *in vitro* to EC and participate *in vivo* in the revascularization of the ischemic area in mice (18). Adipose tissue represents a source for stem cells that is practical, and appealing for autologous cell replacement (16). Furthermore, with the increased incidence of obesity worldwide, an associated risk factor for PVD, subcutaneous adipose tissue is abundant accessible source for adipose derived tissue cells (ADSC). ADSC have the potential to differentiate into bone, cartilage, tendons, skeletal muscle, and fat when cultured under lineage-specific conditions (19). Further it is known that adipose tissue secrete a number of cytokines and it is demonstrated that *in vitro* ADSC can secrete angiogenic cytokines, such as vascular endothelial growth factor (VEGF), for angiogenesis. (16)

The central question in this paper is how adipose derived stem cells can be used as regenerative cell therapy in peripheral vascular disease. First ADSC are described, than the possible mechanism of action of ADSC are mentioned, and finally cell therapy for PVD now and in the future are discussed.

2. Adipose tissue derived stem cells

Stem cells hold a great promise for regenerative medicine because of their ability to self-renew and to differentiate into various cell types. Although embryonic stem cells (ES) have greater differentiation potential than adult stem cells, the former is lagging in reaching clinical applications because of ethical concerns and the potential danger of differentiation into unfavourable cell types, like malignancies through undifferentiated ES (20, 21).

Adult stem cells derived from the bone marrow are the best-studied stem cells, and they are promising candidates in the development of treatments for a wide variety of diseases. In the bone marrow there are three different types of stem cells, namely hematopoietic stem cells, mesenchymal stem cells (MSC), and endothelial stem cells. MSC can differentiate into multiple cell phenotypes, including bone, fat, cartilage, muscle, epithelium, and neural progenitors (16). Although there is no doubt that bone marrow can release progenitor cells that may differentiate into EPC, there are other EPC in the circulating blood that are of non-bone marrow origin (22).

Human MSC have been isolated from adipose tissue, umbilical cord blood, peripheral blood, connective tissues of the dermis, and skeletal muscle (23). Recently discovered ADSC have similarities compared to bone marrow derived MSC. Both represent the stromal cell fraction isolated from an adipose depot, adipose tissue found in specific locations, on the basic of adherence to tissue culture plastic (16). The adipose depot is subcutaneous tissue for ADSC and yellow bone marrow for MSC. Comparing human MSC after culturing derived from adipose tissue and bone marrow showed no phenotypic difference among the stem cells when using a panel of 22 surface antigens (23). Comparing the gene expression profile of the two types of MSC showed that less than 1% of genes are estimated to be differentially expressed between ADSC and bone marrow derived MSC. However, comparing these tissues with fibroblast, they found 25 genes (including fibronectine, extracellular matrix protein 2, glypican-4, isopentenyl pyrophosphate isomerase, nuclear factor I/B, homeobox A5 and homeobox B6) that were overlapping and upregulated (23). ADSC have an equal potential to differentiate into cells and tissues of mesodermal origin, compared with bone marrow derived MSC. Differentiation of ADSC can go, for example, into adipocytes, muscle, cartilage, bone and skeletal.

Clear advantages of ADSC compared to bone marrow derived MSC are the easy and repeatable access to subcutaneous adipose tissue. With the increased incidence of obesity worldwide, subcutaneous adipose tissue is abundant and readily accessible. Approximately 400,000 liposuction surgeries are preformed in the U.S. each year and these procedures yield anywhere from 100 mL to > 3L of lipoaspirate tissue. (14) The simple surgical procedure to isolate makes this tissue source for MSC most attractive for researchers and clinicians of nearly all medicinal subspecialisations (19). The

currently minimal and maximal dose for therapeutic application has not been yet determined, but currently applied doses are in the range of 1 ~ 5 x 10^6 MSC/kg body weight, the lipoaspirate tissue to reach this value is approximately 20 mL (24). A proportion of cells, 2-10%, after the procedure of isolation of the adipose tissue are MSC, whereas bone marrow aspirate yields two to three mesenchymal cells per 100.000 cells (24). It is also know that ADSC in contrary to stem cells derived from peripheral blood expanded more rapidly (more than 10 times within one week). In contrast it is reported that expansion of EPC cultured from the peripheral blood of healthy human volunteers yielded about 5.0 x 10^6 cells per 100 ml of blood. Heterologous transplantation requires 0.5 ~ 2.0 x 10 ^4 human EPC per gram of body weight to achieve satisfactory reperfusion of the ischemic limb (16). This suggests a practical limitation of EPC transplantation. The volume of blood required to extract an adequate number of EPC for autologous transplantation is even higher. The conclusion is that adipose tissue does represent an alternative source of autologous adult stem cells that can be obtained repeatedly in large quantities under local anaesthesia with a minimum of patient discomfort.

The regenerative potential of bone marrow derived MSC and ADSC for cardiovascular diseases is currently under investigation (3). Results of these studies shown that four weeks after intracoronary administration of ADSCs into the infracted myocardium, linker ventricle perfusion, function, and remodelling were substantially improved. This improvement was similar to that observed after intracoronary administration of bone marrow derived MSC. The study did not mention the mechanism of action of the ADSC, but immunohistochemical analysis revealed that the implanted cells indeed differentiated into EC. (25)

It is suggested that there is a homeostatic mechanism that maintains the total adipose tissue volume at a constant level. With rapid weight loss, by for example liposuction, the adipostat operates to return the total adipose tissue volume of an individual back to its initial level. This occurs not only through an increase in the volume of preexisting adipocytes but also through the generation of new adipocytes from a progenitor or stem cell pool. (26)

The phenotypic characterization of ADSC is in progress. Human ADSC express three stem cell markers, CD34, CD133 and ABCG2. These suggest that freshly isolated ADSC contain significant numbers of cells positive for markers of hematopoeietic lineages and endothelial cells (CD45, CD14, CD144, CD34). The markers decrease approximately after 3-5 days in culture. Distinguish between freshly isolated cells from adipose tissue and cultured cells can be made by these markers.(16) By using antioxidants, and a low calcium concentration, growth rate and life span of ADSC can be increased (27).

The question of how ADSC exert their therapeutic effect is certainly not trivial. Investigators have postulated a number of mechanism through which ADSC can be used to repair, and regenerate tissue (26).

During adult life ADSC can contribute to the formation of new vessels in the ischemic area in the limb mainly in two ways, besides the general role of ADSC of generating new adipocytes to maintain the total adipose tissue volume. The first mechanism suggest that ADSC differentiation along a desired lineage and thereby direct incorporation into newly forming sprouts, the so called constructive role of ADSC. Many early studies claimed a significant, up to 50%, direct contribution of grafted stem cells to newly forming vessels in the limbs.

The second mechanism suggests that ADSC contribute to the formation of new vessels, indirectly, by delivering angiogenic growth factors that activate/recruit endogenous vascular cells. The so called instructive role of ADSC (2). ADSC in an injured or diseased tissue secrete cytokines and growth factors that stimulate recovery in a paracrine manner. ADSC are attracting the circulating endogenous stem cells in the blood to the site and promoting their differentiation along the required lineage pathway. Injected ADSC can up regulate the expression of angiogenic cytokines by autocrine and paracrine actions in the ischemic area. (fig 1.)

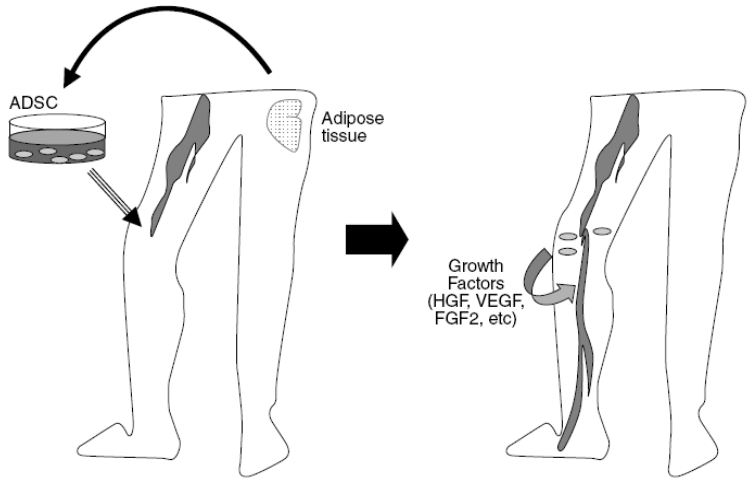

fig. 1 Schema of instructive role of ADSC. The injected cells can secrete a number of growth factors to promote local angiogenesis in the ischemic area (16).

Also other mechanisms are suggested, however they do not seem to be the main mechanisms that contribute to the formation of new vessels. One of these mechanisms suggest that ADSC might provide free radical scavengers, antioxidants chemicals, and chaperone/heat shock proteins at an ischemic state. The result is that toxic substances present in the local environment would be removed. This is promoting recovery of the surviving cells. (23) Other studies have suggested that transplanted bone marrow-derived MSCs can deliver new mitochondria to damaged cells, and thereby rescuing aerobic metabolism. (26, 28)

The relative contribution of the mechanisms depends on the tissue context and the intrinsic characteristics of the ADSC. The direct contribution of grafted cells to newly formed vessels in the limb differs between studies. Mainly early studies claimed a, up to 50%, significant direct contribution of ADSC. Interestingly, studies with EPC shown that although infusion of EPC into ischaemic limbs

can remarkably improve perfusion and recovery from injury, only low numbers of EPC incorporated into the new capillaries can be identified (22). The more recent studies have therefore suggested that ADSC vascular potential regulates may be chiefly, if not exclusively, by the instructive role (2). The supportive function of ADSC may be crucial in ensuring the survival of tissue-residing cells and enhancing blood vessel formation and tissue repair (22).

3. Constructive role of ADSC

The constructive role of ADSC is that ADSC differentiate along a specific lineage pathway to augment repair of damaged or failing organs. The constructive role suggest that stem cells from the bone marrow are on their way to injured tissue, progenitor cells begin the process of differentiation into EC (12). Cytokines and mechanical forces seem to initiate a cascade of events that will lead to some phenotypic features of EC. Shear stress, the mechanical force generated by blood flow, can also effectively induce expression of endothelial specific genes in stem cells. Histone deacetylase activity is essential in this process, which activates p53 and p21 (12).

ADSC have an equal potential compared to bone marrow derived MSC to differentiate into cells and tissues of mesodermal origin such as adipocytes, cartilage, bone, and skeletal muscle. The lineage-specific differentiation into cells of mesodermal origin is well understood on a molecular basis. Among these cells types of mesodermal origin, the differentiation process can be switched, for example, by over expression of lineage-specific transcription factors. Although sparse data exist on ADSC differentiation into tissues of nonmesodermal origin an initial effort has been made to differentiate ADSC into hepatocytes, endocrine pancreatic cells, neurons, cardiomyocytes, hepatocytes, and endothelial/vascular cells. The molecular key events and transcription factors that initially allocate the ADSC to a specific lineage of nonmesodermal origin are almost completely unknown. Decoding these molecular mechanisms is of great interest for a more effective development of novel stem cell therapies. (19)

Factors such as donor age, type (white or brown adipose tissue), and localization (subcutaneous or visceral adipose tissue) of the adipose tissue, type of surgical procedure, culturing conditions, exposure to plastic, plating density, and media formulations might influence both proliferation rate and differentiation capacity of ADSC (14, 19). The source of adipose tissue might influence the long-term characteristics of the fat graft, because it is known that different anatomical localizations of have their own metabolic characteristics, such as lipolytic activity, fatty acid composition, and gene expression profile. Attachment and proliferation capacity are changing during aging, ADSC derived from younger donors compared with older donors are more pronounced. The differentiation capacity is maintained with aging. (19)

Studies with bone marrow derived stem/progenitor cells have been proposed that the circulating stem/progenitor cells in adult organisms are recruited to and incorporate into sites of physiological and pathological neovascularisation. Transplantation of these cells seems to increase recovery of perfusion

and function in models of myocardial and peripheral ischemia. The reported relative contribution of transplanted cells to the endothelium of growing vessels varies widely (29). The more recent reports however shown doubts about the extent to which bone marrow derived stem cells trans-differentiate into organ-specific cells in adult organisms. Studies that used green fluorescence protein (GFP) to locate the bone marrow-derived EPC into endothelial show no incorporation in the endothelial monolayer and tunica media of growing vessels. Nevertheless, these studies found a significant perivascular accumulation of GFP-positive cells in areas of collateral artery growth and capillary growth. These cells stained also positive for some growth factors and chemokines. (29) The suggestion that can be extend from studies with bone marrow derived MSC is that the capability of ADSC to promote vascular growth due to their structural incorporation is irrelevant, but there mechanism of action may be as supporting cells.

4. Instructive role of ADSC

Therapeutic angiogenesis with growth factors in patients with PVD did not show benefit effects. One of the disadvantage from angiogenic cytokines as an intravascular injection is that the practicability is counterbalanced by a short half-life in the circulation (3). Recently, the concept was launched that transplanted cells may instruct host cells by secrete angiogenic growth factors, so that the host cells differentiate into the desired lineage specific pathways (2). ADSC secrete significant amounts of angiogenesis-related mediators at levels that are bioactive, such as VEGF, placental growth factor (PIGF), hepatocyte growth (HGF), basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-β), and angiopoietin. By the secretion of angiogenesis related cytokines the ADSC function as supporting cells. Transplantation of ADSC into an ischemic limb accelerate angiogenesis mainly caused by secretion of growth factors, rather than participation in vessel formation by differentiation (30). This suggests that stem cell transplantation is also “cell-based cytokine therapy”. It is expected that the cytokines are released constantly by the ADSC and thereby have a constant flow.

VEGF induces proliferation, sprouting, migration and tube formation of EC. Although VEGF acts mostly on EC, it has been shown it also binds to VEGF receptors on hematopoietic stem cells (HSC), monocytes, osteoblast and neurons. Besides angiogenesis, VEGF induces HSC mobilization from the bone marrow, monocyte chemoattraction, osteoblast-mediated bone formations and neuronal protection. Furthermore, VEGF stimulates inflammatory cell recruitment and promotes the expression of proteases implicated in pericellular matrix degradation in angiogenesis. VEGF expression in cells is induced by many cytokines including platelet-derived growth factor, epidermal growth factor, basic fibroblast growth factor, and transforming growth factors and VEGF itself. (30)

However besides the positive effects of VEGF in relation to PVD mentioned above there are also some negative effects in relation to atherosclerosis. Atherosclerotic plaque progression is associated with inflammatory angiogenesis through increased secretion of VEGF. Plaque angiogenesis results in atherosclerotic plaque growth, increased plaque instability, and an increased risk of intraplaque

haemorrhage. These factors contribute to the growing instability of the atherosclerotic plaque. Experimental atherosclerotic changes can be induced with VEGF, and experimental atherosclerotic changes can be reduced with antiangiogenic therapy. In animal models it was found that VEGF mediates the progression of arteriosclerosis. However, recent results have suggested that the angiopoietins that bind and activate tyrosine kinase recpetors on EC, could actually prevent some of the inflammatory changes in the vessel wall. Further investigations must be done for safety reasons. (31)

VEGF and PIGF are structurally related. PIGF stimulates angiogenesis and collateral growth in ischemic heart and limb at least with a efficiency comparable to VEGF. In the formation of both the blood vessels and lymphatic vessels, VEGFs and angiopoietins appear to have complementary roles. The VEGF system appears to play a key role in vessel sprouting and new vessel initiation, whereas Angiopoietins play a role in the remodelling/maturation phases. (32)

FGF binds high-affinity receptors possessing tyrosine kinas activity as well as transmembrane heparin sulphate carrying core protein syndecan. The FGFs are potent regulators of cell proliferation, differentiation, survival, adhesion, migration, motility, and apoptosis (3). Furthermore, the biological effects of FGF administration includes cardio protection, angiogenesis, vascular remodelling, and cardiac remodelling (3). In animal models with hindlimb ischaemia FGFs were shown to stimulate angiogenesis. The effects of FGF and VEGF are suggested to be synergistic, which suggest that these two proteins may act complementarily.

5. Current cell therapy in PVD

The concept of cell-based therapy has moved remarkably fast from bench to bedside. First clinical studies are already published. Experiments with bone mononuclear fraction of bone marrow and/or peripheral blood in CLI patients as cell-based therapy have been published, 5 years after the description of EPC, all studies were performed in an autologous setting (2). Most of these studies enrolled a limited number of CLI patients or were case studies and did not include a placebo arm. None of these studies had a double-blinded randomized design and the follow-up period was relatively short (mean approximately 7.9 months). (33) Additional laboratory studies on cell-based revascularization has yielded important new insights. The first clinical experiences has raised new questions that could only be solved by additional preclinical research. These data are needed for full exploitation of the benefits of cell therapy. Experiments with ADSC based therapy for PVD patients has not yet been published.

During the experiments the animal model that should be used for a preclinical PVD model, should represent the human disease. The most frequently used animal model is the rodent. In rats there is a relatively abundant pre-existing collateral network. The mouse reserve system is more closely like the human. Methods used to provoke ischemia in animal are always acute, in contrast with the usual chronic nature of the disease in humans. Some mouse strains (BalbC) are characterized by a less

elaborate collateral network, meaning that there is a slower and less extensive spontaneous flow recovery and a higher incidence of tissue necrosis following ligation. Therefore, BalbC mice have been used as a model for CLI, while strains with high collateral reserve, such as C57B1/6, have been used as a model for IC. (2)

In cell therapy the vascular regeneration is in most PVD patients not sufficient. CLI patients that are being considered for revascularization, do also have muscle damage. The ideal therapy in these patients would be angiomyogenesis, a yield combining vascular and muscular regeneration. (2)

Studies involving bone marrow derived stem cell therapy in patients with PVD by transplantation so far have showed a relatively low associating rate of adverse event. The long-term safeties of these kinds of therapy still have to be solved. A concern is raised from experimental studies where bone marrow cells and EPC transplantation were associated with acceleration of atherosclerosis. (3, 4) Another field of caution is the involvement of stem cells in carcinogenesis. To date, the role of EPCs in tumor angiogenesis has been clearly demonstrated by several groups (3). It is showed that bone marrow derived cells transduced with an anti-angiogenic gene can restrict tumor growth in mice (3). The knowledge that EPCs are critical for tumor neovascularisation is being used to explore new strategies to inhibit tumor growth by suppressing progenitor cell number and function. Although it is not known whether local administration of exogenous EPC may increase tumor neovascularisation, this issue needs careful consideration before moving to clinical applications. Also it has to be investigated of these disadvantages for EPC and bone marrow derived MSC also count for ADSC. It is known that the ADSC are pro-angiogenic and anti-inflammatory, and this is maybe an advantage of using ADSC as cell therapy in stead of EPC or bone marrow derived MSC to treat PVD.

In all contexts, it is important to consider the potential use of both autologous and allogeneic ADSC. Autologous ADSC offer advantages from regulatory, histocompatiblility, and infectious perspectives. Recent studies support further evaluation of allogeneic ADSC transplantation. The hypothesis that transplanted allogeneic ADSC will not elicit a robust immune response and subsequent rejection needs independent and comprehensive test. (26)

6. Conclusion

Although pioneering clinical experience with stem cell-related therapy seems promising, it is too early for general clinical use of this technique, since many questions remain unanswered. Indeed, while questions about safety, dose, and administration route/timing/frequency are the first ones to be addressed when designing a stem cell-based clinical approach, there is accumulating evidence from recent (pre-) clinical studies that other issues may also be at stake.

The choice of ADSC to use as stem cells for cell therapy in PVD hold a good promise. Compared with bone marrow derived MSC they show no phenotypic difference, although there are some clear advantages of ADSC compared to bone marrow derived MSC. Adipose tissue represent a source of autologous adult stem cells that can be obtained repeatedly in large qualities under local anaesthesia

with a minimum of patient discomfort. The main benefit of ADSC above bone marrow MSC is that they can be easily harvested from patients by a simple, minimally invasive method and also easily cultured. Studies using stem cell therapy for cardiovascular diseases shown a similar effect of bone marrow derived MSC and ADSC.

The precise mechanism of the cell therapy with ADSC is still unclear. However, the more recent studies are suggesting that ADSC vascular potential may be chiefly, if not exclusively, by the instructive role. Cultured ADSC secrete significant amounts of angiogenesis-related mediators at levels that are bioactive, such as VEGF, PIGF, HGF , bFGF, TGF-B, and angiopoietin. By the secretion of angiogenesis related cytokines the ADSC function as supporting cells . Transplantation of ADSC into an ischemic limb accelerates angiogenesis mainly caused by secretion of growth factors, rather than participation in vessel formation by differentiation. However besides the positive effectives of VEGF in relation to PVD mentioned above there are also some negative effects in relation to atherosclerosis. Further investigations must be done for safety reasons. The long-term safety of these kinds of therapy still have to be investigated. All therapies using pro-angiogenic strategies have the disadvantages of tumor neovascularisation. Although it is not known whether local administration of exogenous EPC may augment tumor neovascularisation, this issue needs careful consideration before moving to clinical applications of ADSC cell therapy to treat PVD. Another field of caution is the involvement of ADSC in carcinogenesis. Further the long-term survival of engrafted cells in the absence of a normal supportive tissue environment should be well considered.

Trials performed to date suggest that cell therapy could serve as a much needed new therapeutic modality for treatment of symptoms of advanced PVD. However, cell therapy trials with bone marrow derived MSC to date have enrolled small numbers of subjects, have often been unrandomized, and have largely been performed to investigate feasibility and safety of these approaches. The optimum cell preparation, delivery, dose, and frequency of administration are yet to be established.

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