

Therapeutic potential of adipose-derived stem cells in vascular growth and tissue repair

Soon Jun Hong^{a,b,c,e}, Dmitry O. Traktuev^{a,b,c} and Keith L. March^{a,b,c,d}

^aKrannert Institute of Cardiology, ^bIndiana Center for Vascular Biology and Medicine, ^cIndiana University School of Medicine, ^dR.L. Roudebush Veterans Affairs Medical Center, Indianapolis, Indiana, USA and ^eKorea University Anam Hospital, Seoul, Korea

Correspondence to Keith L. March, MD, PhD, Indiana Center for Vascular Biology and Medicine, 975W Walnut Street, IB 441, Indianapolis, IN 46202, USA
Tel: +1 317 278 0130; fax: +1 317 278 0089;
e-mail: kmarch@iupui.edu

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Purpose of review

Adipose-derived stem cells (ASCs) are readily available from autologous adipose tissue and have been demonstrated to provide significant potential for tissue rescue from, or repair of, damage in multiple animal models. These include models of myocardial infarction, heart failure, hind limb ischemia, and inflammatory conditions. Early clinical studies have now extended testing of the effects of ASC into patients. This review highlights some of the key reports underlining the potential of ASCs, focusing particularly on diseases involving the cardiovascular system, vascular growth, and tissue repair.

Recent findings

Clinical applications of ASCs have begun to show early safety results and promising possibility of efficacy in patients with a range of diseases, including acute myocardial infarction, peripheral vascular disease, and soft and bony tissue defects including cranial bone loss, Crohn's-related fistula, and skin wounds. These effects are importantly based on the secretion of trophic and survival factors by these cells and by their participations in the growth and remodeling of blood vessels. These results suggest that ASCs could be a valuable therapeutic option in vascular growth and tissue repair in various clinical settings.

Summary

ASCs may ultimately represent a valuable therapeutic option in tissue rescue and repair based on their ready availability, proangiogenesis and antiapoptotic factor secretion, immunomodulatory effects, and capacity for multilineage differentiation and ready expansion.

Keywords

adipose-derived stem cells, adipose-derived stromal cells, regenerative medicine, tissue engineering, vascular growth

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Introduction

The concept of using stem and progenitor cells isolated from the tissues of adults to treat patients with a variety of diseases is gaining momentum. There has been particular interest in studies of cell therapy for diseases of the cardiovascular system. The enthusiasm of using progenitor cells in such clinical fields has been supported by experimental data obtained in rodent, porcine, and canine models of these diseases. Initial studies involved skeletal myoblasts [1–3], bone marrow-derived mesenchymal stromal cells [4–15], and various subtypes of blood-derived endothelial progenitor cells (EPCs) [15–20]. However, during the past several years, the possibility of therapies employing cells residing in the 'stromal vascular fraction' of adipose tissue has attracted progressive attention. For many years, the cells residing in the stroma of the fat were considered to be preadipocytes;

however, more recent studies [21–23] have revealed a much wider range of functional properties *in vivo*, as well as differentiation potential *in vitro*. One of the significant practical factors supporting the therapeutic use of adipose-derived stem cell (ASC) is the potential to readily obtain an autologous preparation of these cells for injection within the timeframe of 1–2 h. Depending on the method of cell isolation and harvesting, approximately 10^5 – 10^6 ASCs per gram of tissue can be obtained, and if required, these cells are easily and rapidly expanded.

Adipose-derived stem cells: cousins of bone marrow-derived marrow stromal cells

ASCs are mesenchymal cells that share many properties in common with the bone marrow-derived cells known as mesenchymal stem cells as well as marrow stromal cells (MSCs) [24,25]. Recently, several studies have been

performed directly comparing characteristics of ASCs with those of the bone marrow-derived MSCs (BM-MSCs). It has been shown that the surface immunophenotype of ASCs is broadly similar to that of BM-MSCs: both are positive for CD10, CD13, CD90, and CD106. Whereas CD34 antigen is not generally expressed on cultured BM-MSCs [26–30], freshly isolated human ASCs (hASCs) express CD34 antigen, which is lost over a period of days in culture [31]. It is most likely that BM-MSCs also express CD34 antigen *in vivo* [32], but the necessity of culturing bone marrow over a 2–3-week time frame to obtain BM-MSC colonies leads to the loss of this marker. Similar to BM-MSCs, ASCs are capable of differentiating into multiple cell lineages. Many laboratories have demonstrated human and murine ASC differentiation into osteoblasts and chondroblasts as well as adipocytes, while some have also described differentiation into neurons, smooth muscle cells, skeletal myocytes, cardiomyocytes, and endothelial cells [33–45]. A key potential advantage of ASCs for therapeutic applications is based on their much greater numerical availability for harvest from adipose tissue, which potentially renders culture expansion unnecessary, as well as their ease of harvest by a minimally invasive liposuction. Indeed, most clinical cases and studies testing ASC in humans to date have employed autologous preparations derived by enzymatic and mechanical processing of adipose tissue to obtain cell pellets rich in ASC, without the need for subsequent culture. This approach would seem to restrict the potential for harmful transformation events during ex-vivo culture while facilitating the regulatory approach to such trials. It is interesting to note, however, that the use of autologous cells in these trials raises the potential for variability of the therapeutic cellular agent based on donor biology (e.g., age and disease status). Although studies have revealed that ASC quantity available from liposuction depends on donor age, BMI, and tissue harvest site [46], very little effort to date has focused on qualitative differences among the activities of ASC as related to donor (patient) sourcing.

Vascular growth and cardiac tissue rescue by adipose-derived stem cells

Our early observations that ASCs secrete significant quantities of angiogenic and antiapoptotic factors, including vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) [31], led to a series of in-vivo studies focused on evaluating the therapeutic potential of the cells based, in particular, on their paracrine and angiogenic effects. Intramuscular as well as intravenous injections of approximately 5×10^5 hASCs have been demonstrated to successfully revascularize ischemic hind limbs of immunocompromised mice [31,47,48]; moreover, the effect of hASCs has been shown to be superior

in terms of salvaging of ischemic hind limb in comparison with human MSCs [48]. The findings of positive effects of ASCs on blood flow as well as ischemic tissue salvage have been replicated by numerous groups with complementary experimental designs [31,47–51]. These robust effects have fostered investigations of the roles of ASC in vascular biology, as well as the design of additional preclinical studies directed towards ischemic tissues, and ultimately clinical trials in this area.

Intramyocardial injection of hASCs has been found to preserve heart function and augment local angiogenesis following myocardial infarction (MI) in multiple studies [52–58]. As in hind limb studies, it appears that a predominant mechanism of action of ASC involves provision of beneficial trophic factors [52]. In multiple reports, ASC administration has led to improvement in left ventricular ejection fraction and fractional shortening after MI, although left ventricular remodeling and dilation were limited in the ASC group when compared with the physiological saline group [52]. Similar improvements in left ventricular function and perfusion via angiogenesis following MI have been reported in porcine experiments that demonstrated ASC engraftment in the infarct region 4 weeks after intracoronary cell transplantation [55]. Overall, ASC treatment of animals with acute MI has demonstrated improvement of global ejection fraction of about 7.4% (3.4–12%), as well as improved myocardial perfusion [52,54–57]. Histological examination of the myocardial tissue revealed increases in tissue neovascularization, reduction of cardiac myocyte apoptosis, and decrease in inflammation in the region of ASCs injection [46]. Additionally, it has been observed that injection of allogeneic ASCs led to local recruitment of both new neural sprouts [52] and endogenous progenitor cells of heart or bone marrow origin [59]. However, the observation of ASC differentiation into cardiomyocytes *per se* appears to be an exception rather than the typical finding. Within experiments such as these, the relative contribution of ASC via antiapoptotic effects vs. via proangiogenic mechanisms remains to be determined.

Wound healing by adipose-derived stem cells

Several studies have evaluated the potential therapeutic effects of ASCs on wound healing. Local implantation of ASCs has been found to be effective in supporting epidermal healing in full thickness skin wounds of pigs [60], as well as in rats, in which the survival area of ischemic skin flaps was significantly increased by local injection of autologous ASCs [61]. Topical administration of ASCs placed in type I collagen sponges also accelerated the skin wound healing process in rats with skin ulcers [62]. Wound healing in diabetic mice with skin ulcers was more effective with topical administration of autologous ASCs [63]. Increase in tissue vascularization

was reported in each of these cases and is thought to be an important component of this activity.

Proposed mechanisms of adipose-derived stem cells in tissue repair and vascular growth

Several hypotheses explaining the therapeutic effects of ASC on ischemic or infarcted tissues have emerged: secretion of angiogenic/antiapoptotic factors [31,48,49, 64[•],65,66,67^{••}] that can stimulate angiogenesis (in-growth of host vasculature into the ischemic region) and locally preserve or rescue ischemic tissue from damage; secretion of recruitment factors that enhance availability of progenitor cells, either locally or at a distance (e.g. EPCs released into the blood stream); differentiation of ASC into vascular cells with integration into the vasculature [50,51,68^{••}] as a functional component of neovessels; and differentiation into parenchymal cells such as cardiomyocytes [33,69].

The hypothesis that paracrine effects play dominant roles in the therapeutic function of ASCs has been supported by multiple studies *in vitro* and *in vivo*. We have previously demonstrated that ASCs secrete multiple angiogenic [VEGF, HGF, basic fibroblast growth factor (bFGF), basic nerve growth factor (bNGF), angiogenin, and angiopoietin-1], inflammatory [interleukin (IL)-6, IL-8, IL-11, IL-17, monocyte chemoattractant protein (MCP)-1, and MCP-2], and mobilizing [granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage colony-stimulating factor (M-CSF), and stromal cell-derived factor 1 (SDF-1)] factors [31,64[•],67^{••},70, 71[•]]. Antioxidant chemicals, free radical scavengers, and heat shock proteins have also been described as produced by ASCs, fostering a protected environment for host cell recovery from insult [27]. The necessary role of paracrine secretion in mediating the *in-vivo* effects of ASC was specifically supported by a study [65] demonstrating that blockage of HGF synthesis markedly reduced the ability of ASC to promote angiogenesis in ischemic muscle tissue, whereas in a complementary study [67^{••}], systemic neutralization of SDF-1 activity following ASC administration also diminished blood flow recovery in ischemic tissue, in parallel with a reduction of circulating EPC levels.

Recently, we found that ASCs are primarily located in the walls of adipose microvasculature, possess many characteristics of pericytes [64[•]], and are capable of stabilizing endothelial networks *in vitro* as well as robustly synergizing with endothelial cells to participate in the *in-vivo* formation of new vessels that connect with host vasculature, conduct blood flow, and exhibit network stability for several weeks [68^{••}]. This synergistic assembly of functional vessels from ASC and endothelial cells, two non-transformed and readily available human cell components,

coupled with the formation and remodeling of a vascular network in the context of physiologic flow, has supported the hypothesis that ASC function not only by secretion of active factors, but also by providing ‘building blocks’ or ‘hardware’ for vascular structures. This novel model of postnatal vasculogenesis also permits study of mechanisms underlying this process and has led us to investigate the possibility that a synergy between ASC and endothelial cells might provide a practical approach to tissue vascularization for implants, transplants, or regional ischemia. Even in the context of this vascular assembly, the paracrine activity of ASC plays an important role: interruption of platelet-derived growth factor-BB (PDGF-BB) signaling within subcutaneous implants carrying mixtures of endothelial cells and ASC prevented these cells from assembling into the multilayer vessels [68^{••}].

The ‘differentiation’ hypothesis of ASC is primarily based on *in-vitro* studies identifying that rodent and hASCs were able to differentiate into skeletal myocytes [35,38] and cardiomyocytes under specific conditions [33,34,44,45,72–75]. However, there has been little data supporting the capability of ASCs to differentiate into any of these cell types *in vivo*; moreover, the frequency of cardiomyogenic differentiation appears to be rather low, even *in vitro*. The potential of ASCs to contribute to vascular formation by differentiation into endothelial cells has been suggested by several *in-vitro* and *in-vivo* studies [47,50,51]. However, the lack of clonal purification and the fact that freshly isolated or minimally expanded populations of ASCs are contaminated with endothelial cells [31,50,51] limit interpretation of some of these data.

Adipose-derived stem cells in clinical trials

Positive experimental data, accumulated from the studies outlined above in experimental models of cardiovascular disorders, have prompted several clinical studies. Although the full publication of data from many of these trials is pending, early information from selected trials has been presented recently. The first study to be reported in the context of human peripheral vascular disease described multiple intramuscular injections of autologous cultured ASCs in six patients with Buerger’s disease. Endpoints of this study included maximal and pain-free walking distance, toe-brachial and ankle-brachial pressure indices, laser Doppler cutaneous flow, and ulcer healing. After 24 weeks of treatment, autologous ASCs had been well tolerated and improved clinical performance while reducing resting pain in these patients [International Federation of Adipose Therapeutics and Science (IFATS) meeting, 2009]. Intramuscular injection of ASCs also has recently shown clinical efficacy in patients with diabetic mellitus foot and arteriosclerosis obliterans. Rest pain score, peak walking time, and vascular collateral networks improved significantly 6 months after the ASC

injections without any complication (IFATS meeting, 2009).

On the basis of the collected data, described above, that ASC could significantly improve function of cardiac muscle in experimental disease models, key clinical studies have recently been initiated. Serruys and colleagues have now completed enrollment in the APOLLO (3D adipose-derived stem-cell transplantation in the treatment of patients with an acute ST-elevation myocardial infarction) trial, the first-in-man test of ASC transplantation into patients with an acute ST-elevation MI. This study will evaluate the safety and feasibility of ASCs delivered via intracoronary route in a dose-escalating strategy. The PRECISE (3D adipose-derived stem-cells in the treatment of patients with nonrevascularizable ischemic myocardium) trial, performed by Fernandez-Aviles and colleagues, is a complementary randomized clinical trial of ASC administration in the treatment of symptomatic nonrevascularizable ischemic myocardium and is also in progress; in this trial, intramyocardial ASC delivery is being performed under the guidance of NOGA-left ventricle (LV) mapping (Biosense-Webster, Tirat Macarmel, Israel) (4th International Conference on Cell Therapy for Cardiovascular Diseases, 2008).

Issues to be resolved: fresh vs. cultured and autologous vs. allogeneic

Although many lines of evidence support the promise of ASC-based therapies with respect to vascular growth and tissue repair in ischemic tissues, certain key questions remain outstanding with respect to optimizing the implementation of such therapies. Indeed, uncertainty with regard to their answers must be borne in mind as early clinical results emerge, as it is possible that current limitations of knowledge could, in fact, underlie any suboptimal outcomes. Examples of such key questions include the selection of fresh ASC (with or without purification from the heterogeneous population of cells derived from initial processing) vs. culture-expanded ASC; choice of autologous vs. allogeneic ASC for particular treatment approaches; and choice of optimal delivery approaches to provide ASCs to each particular target tissue of interest, with appropriate delivery distribution and safety. With regard to the use of fresh vs. cultured ASC, the more prominent heterogeneity of fresh ASC preparations may be either a positive (if more than one complementing cell is helpful in conferring therapeutic benefit) or a negative, if particular active populations would be amplified by culturing. On the contrary, the culture expansion process itself may be the occasion of undesirable alterations of ASC, as in a study [76] that showed expanded hASCs to transform into tumors in immunodeficient mice; conversely, another

study [77] demonstrated high genomic stability of ASCs.

The complementary choice of autologous vs. allogeneic ASCs is also critical in clinical trial design. It has been noted that ASCs, similar to BM-MSCs, do not express human leukocyte antigen (HLA)-DR protein [46]. Accordingly, they are significantly less immunogenic than other cell types. ASCs do not provoke a mixed lymphocyte reaction when cocultured with allogeneic peripheral blood monocytes [27,29,78], do not stimulate immune response in a recipient, and suppress a cytotoxic T-cell response *in vivo* [27], thereby allowing allogeneic or potentially even xenogeneic transplantation. Interestingly, the immunomodulatory effects of ASCs have been observed even after their differentiation into other cell types [79,80]. On the basis of these observations, and in analogy to trials conducted with BM-MSCs, ASCs have been applied to patients with steroid-refractory graft-versus-host disease (GVHD) [81]. Acute GVHD was found to completely subside after intravenous injection of ASCs in 83% of treated patients evaluated at a median follow-up of 40 months [81]. Also of particular interest in the context of solid organ transplantation, GVHD after liver transplantation has been suppressed by infusion of unrelated ASCs into the recipient [82]. If subsequent studies continue to bear out the general feasibility of ASC allotransplantation, an option of selection of well characterized samples of stored, expanded ASCs for 'off-the-shelf use' will become more realistic.

Conclusion

Tissue engineering, including limitation of damage and promotion of organ repair, can be achieved by ASCs in a number of test systems, which have demonstrated their capacity to facilitate angiogenesis, limit apoptosis, promote vascular growth, and to provide immunomodulatory effects. ASCs represent remarkably pragmatic therapeutic candidates to augment vascular growth and tissue repair, as ASCs can be readily isolated in large quantities and can be harvested by a minimally invasive liposuction. ASCs can also be differentiated into a range of cell types using predictable and reproducible methodologies and exhibit potentially therapeutic effects when transplanted to either an autologous or allogeneic host. Ongoing and future work will illuminate the range of possibilities for translation of this promising population of cells into clinical utility.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 140).

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