Adult Stem Cell Therapy for Injured Solid-Organ Tissue (with Emphasis on Cardiac Tissue Repair)

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Abstract: Adult hematopoietic tissue-derived stem cells are primarily used for hematopoietic reconstitution in patients with malignant lympho-hematopoietic disorders undergoing stem cell transplantation. Their therapeutic use for solid-organ tissue repair such as cardiac tissue is a novel treatment strategy. Besides hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), endothelial progenitor cells (EPCs) and even solid-organ tissue resident stem cells (e.g. cardiac stem cells [CSC]) are known to contribute to solid-organ tissue repair. Stem cell delivery for cardiac tissue repair is by transvascular or intramyocardial injection. Myocardial tissue engineering takes advantage of biocompatible materials as stem cell carriers (scaffolds). Stem cell concentration at the site of tissue injury and engineering the stem cell microenvironment are additional components that determine treatment outcome. Mechanisms that explain how stem cells generate solid-organ specific cells include nuclear re-programming (transdifferentiation), cell fusion, and paracrine secretion of transplanted cells (e.g., MSCs). One preferred therapeutic concept is the stepwise induction of neovascularization followed by activation of the solid-organ resident stem cell pool. Cardiac tissue repair in patients with acute myocardial infarction is currently at the forefront of clinical stem cell treatment research. The outcome of major clinical trials will be discussed.

Keywords: Adult stem cells, tissue repair, cardiac stem cells, myocardial tissue engineering, regenerative medicine.

INTRODUCTION

Adult stem cell-induced solid-organ tissue repair emerges as a new discipline with emphasis on repair of injured tissue, replacement of non-functioning tissue, and generating new tissue. Adult stem cell/cell therapy products are considered medicinal products or drugs, and, as is practiced for example in Germany, their manufacturing is regulated according to the German Drug Law (Deutsches Arzneimittelgesetz). The European Medicines Agency (EMEA) oversees the quality, safety and efficacy of advanced therapy medical products including cellular products [1]. In the United States modified stem cell/cell therapy products are registered at the Food and Drug Administration (FDA), and manufacturing is regulated by the Foundation for the Accreditation of Hematopoietic Cell Therapy (FAHCT), the American Association of Blood Banks (AABB) and the Clinical Pathology Accreditation (CPA). Per FDA regulation therapeutic cell products are processed in so-called Good Manufacturing Practice (GMP) cell therapy laboratories.

Stem cells in any adult tissue are defined as being clonogenic, having self-renewal capacity throughout lifetime and giving rise to terminally differentiated cells of various cell lineages. Two basic cellular treatment strategies are conceivable: either using embryonic stem cells (or equivalent) that are differentiated ex vivo into heart tissue-specific cells prior to *in vivo* transplantation, or using the patient's own adult stem cells that are, with or without *ex vivo* manipulation, transplanted *in vivo* into heart muscle tissue. Adult lympho-hematopoietic stem cells are the most thoroughly characterized adult progenitor cells, mostly because of their easy accessibility, and more than 40 years of experience with their clinical use for transplantation to treat lymphohematopoietic disorders. This review focuses on the therapeutic use of adult stem cells with emphasis on cardiac tissue repair.

COMPONENTS OF ADULT STEM CELL THERAPY

The concept of adult stem cell/cell therapy is based on essentially four components:

1. stem cell/cell source,
2. cell delivery,
3. cell concentration at the site of tissue injury, and
4. stem cell microenvironment.

Stem Cell Source

For practical and therapeutic purposes adult stem cells are subdivided into hematopoietic tissue- and non-hematopoietic tissue-derived. Hematopoietic tissue-derived stem cells (HSCs) originate in the subendosteal and perivascular niches of bone marrow (BM), in the circulating blood and marginal blood pools, and in umbilical cord blood (UCB). Non-hematopoietic tissue-derived stem cells are located inside solid-organ tissue such as heart muscle. HSCs have the major advantage of being easily accessible by BM aspiration, large volume apheresis, or as discarded UCB. Another advantage is the large quantity of stem cells

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available for harvesting being in the range of up to 10^8 per harvesting procedure.

Besides HSCs, ex vivo expanded mesenchymal (stroma-derived) stem cells (MSCs) are being increasingly used in clinical tissue repair trials. MSCs are a non-hematopoietic, adherent stem cell population residing in hematopoietic tissue, mostly in BM, but they are also found in peripheral blood (PB) and UCB. MSCs are clonogenic and can be easily expanded in culture giving rise to fibroblastic colonies. Tissue injury or inflammation attracts MSCs to facilitate tissue repair by releasing chemokines/cytokines [2]. Another hematopoietic tissue-derived cell population involves EPCs that can easily be harvested in large quantities by continuous-flow apheresis and locally injected for therapeutic vasculogenesis. The total number of EPCs harvested by continuous-flow leukapheresis and available for treatment after CD133 selection is in the range of forty millions [3, 4].

In an experimental setting a rare Multipotent Adult Progenitor Cell (MAPC) population residing in BM has been identified differentiating into tissue of all three germ layers [5].

Non-hematopoietic solid-organ tissue-derived stem cells of endodermal, mesodermal and ectodermal origin are less well characterized. Difficulty to harvest (biopsy), small amounts obtained, and the need for *ex vivo* expansion (digestion, clonal expansion, feeder layer, etc.) make solid-organ tissue cells less suitable for clinical cell therapy. On the other hand, solid-organ tissue resident stem cells have the advantage of being already programmed *in vivo* to differentiate into functional cells (e.g., cardiomyocytes).

Solid-organ tissue stem cells have been identified in almost any organ system including mammary gland stem cells (mammospheres), cardiac stem cells (CSCs), skeletal myoblasts (satellite stem cells), and neural stem cells. CSCs (also named multipotent cardiac stem cells, cardioblasts, cardiac SP cells) in particular are phenotypically characterized as Lin$^-$ c-kit$^+$, Sca-1$^+$, CD31$^-$, MDR1$^+$. They are self-renewing and multipotent, and they express the cardiac transcription factor *Isl1*, and the multidrug resistance ABCG2 cassette transporter gene. CSCs are clonogenic (cardiospheres), expressing c-kit and myosin heavy chain [6-8]. Using Carbon-14 incorporation during the atmospheric nuclear bomb testings in the 1960-ies as a marker, the annual turnover of cardiomyocytes was calculated to be 1% at age 25, and 0.45% at age 75. Less than 50% of cardiomyocytes are exchanged during a normal life span [9].

Up to now, the preferential stem cell source used in a clinical setting is HSCs and MSCs. This also includes EPCs that are usually not identified when transplanted as BM- or PB-derived mononuclear cells. *Ex vivo* processed skeletal myoblasts and adipocytes are explored in a more experimental clinical setting [10, 11].

Stem Cell Delivery

The easiest way to direct stem cells to the site of tissue injury is by *in vivo* mobilization from hematopoietic tissue into PB using cytokines (e.g., granulocyte colony stimulating factor [G-CSF]) [12]. Transvascular cell delivery into the coronary artery through an angioplasty balloon has been widely used. Alternatively, the intramyocardial injection of cells either as transepicardial injection (requires sternotomy) or as transendocardial injection (percutaneous femoral approach) is the more efficient approach with 11% of cell injected being retained in the border infarct zone versus 2.6% of cells injected intracoronarily [13].

The timing of intracoronary BM-derived stem cell infusion in AMI patients is between 3 and 10 days after reperfusion, best at day 5 or 6 after infarction.

Myocardial tissue engineering (MTE) is an evolving field using bio-compatible and bio-degradable materials as cell carriers (scaffolds) that are transplanted or injected into the site of tissue injury. There are three different approaches of MTE:

1.   *in situ* engineering with injection of cells together with an injectable biomaterial (e.g., hydrogel),

2.   injection of a biomaterial alone, and

3.   *ex vivo* tissue engineering in a bioreactor with subsequent implantation.

Biomaterials include synthetic or natural polymeric materials, or a combination of both. The grafted tissue, cellular or acellular, will eventually direct new tissue formation as the cells integrate with the native tissue and the biomaterial degrades over time [14].

The most radical experimental approach of cardiac tissue repair has been reported by H.C. Ott and colleagues using a decellularized and perfused heart matrix as a platform to engineer a bioartificial heart. The natural heart matrix was re-cellularized with neonatal cardiac cells through intramural injection. The heart construct could generate pump function equivalent to 25% of a 16 week fetal heart function [15].

One of the central challenges of cell-based therapy for regenerating specific heart components is guiding transplanted cells into a functional syncytium within the existing three-dimensional structure of muscle tissue, coronary vasculature and conduction system.

Cell Concentration at the Site of Tissue Injury

In a highly publicized paper Orlic's group was able to successfully repair infarcted myocardial tissue in an experimental setting of stem cell mobilization treatment with G-CSF and stem cell factor [16]. Later translational and clinical studies could not confirm those early data [12]. The reason for clinical failure was a 250-fold increase in stem cell concentration at the site of tissue injury at any given time in Orlic's study that is out of reach in a clinical setting. The one time median number of stem cells injected in major clinical cardiac tissue repair trials was 27 x 10^4 CD34$^+$ cells [8]. Keeping in mind that only 11% of intramyocardially injected cells are retained in muscle tissue [17], the median number of injected stem cells actually residing in the border zone of infarcted tissue is reduced to 27 x 10^3 CD34$^+$ cells. The corresponding number of CD34$^+$ cells for hematopoietic engraftment is 4 orders of magnitude higher.

Stem Cell Microenvironment

The stem cell microenvironment, also called stem cell niche, aides to the control of stem cell renewal, differentiation, quiescence and apoptosis. Such control is

maintained through short-range intercellular signals such as chemokines and cytokines, extracellular matrix, neighboring cells (e.g., osteoblasts) and physical stimuli (e.g., fluid shear stress) [18]. Whereas the subendosteal and perivascular hematopoietic stem cell niche is the best characterized, other stem cell niches have been described for muscle tissue, CNS, intestinal epithelium, hair follicle and others. The vascular niche in particular consists of sinusoidal endothelial cells which provide a platform for survival and differentiation of stem and progenitor cells. As shown for the hematopoietic system stem cell engraftment and differentiation is dependant on vascular endothelial growth factor receptor-2 mediated regeneration of sinusoidal endothelial cells after tissue injury [19]. A similar mechanism could apply to cardiac tissue repair.

MECHANISMS OF HOW STEM CELLS/CELLS CONTRIBUTE TO TISSUE REPAIR

The various concepts of how stem cells and even end-differentiated cells contribute to tissue repair are controversial and discussed as such in the literature [20].

There are essentially five models explaining how hematopoietic tissue-derived stem cells or cells are integrated into or contribute to the generation of solid-organ tissue *in vivo*:

1. Each tissue has its own circulating stem cell pool contributing to lifelong tissue homeostasis. To underline the close relationship between circulating stem cells and stationary solid organ tissue stem cell pools, it is noteworthy that hepatic oval cells as part of a solid organ tissue resident stem cell pool share the same phenotype with circulating stem cells being $CD34^+$ $CD90^+$ c-kit^+ $CXCR4^+$ [21]

2. *Ex vivo* expanded BM-derived MAPCs, when transplanted into a host, differentiate *in vivo* into epithelium of liver, lung and gut besides generating blood cells [5]. It is therefore conceivable that a small reservoir of primitive stem cells is available throughout lifetime to replenish local stem cell pools in case of tissue damage or exhaustion.

3. Adult stem cells that differentiate inside their own tissue may deviate from their preprogrammed pathway to generate under conditions of stress (e.g., tissue injury) solid-organ cells of a different tissue. This process is called 'transdifferentiation' or 'nuclear re-programming'. The reverse mechanism is called 'de-differentiation' where more differentiated cells regain stem cell status to eventually differentiate into another tissue lineage. Using the Y-chromosome as a marker in a sex-mismatched HSC transplant setting, numerous reports over the past 8 years indicate that adult stem cells or their progeny derived from hematopoietic tissue (BM, PB, or UCB) not only generate blood cells but also, at a much lower frequency, solid organ-specific cells [20]. Similarly, after transplantation of a female heart into a male recipient, up to 10% of transplanted cardiac tissue cells contained the recipient's Y-chromosome [22].

4. Adult stem cells derived from hematopoietic tissue or their progeny may fuse with solid organ tissue cells to generate hybrid cells. On a clinical level we identified solid organ tissue cells containing both, donor-derived and host-derived genetic material indicating fusion [23]. The physiological purpose of adult cell fusion is speculative. As outlined by Helen Blau fusion could be a means by which cells 1) deliver healthy genetic material to dying cells (rescue function), 2) supply cells with new genes (repair function), or 3) correct genetically defective cells such as in muscular dystrophy (gene replacement). Fusion could even be considered a basic mechanism for keeping the adult cell systems intact throughout our lifespan [24].

5. Hematopoietic tissue-derived stem cells injected locally near injured tissue may act as trophic mediators *via* secretion of various angiogenic, antiapoptotic and mitotic/differentiating factors thus activating the intrinsic solid-organ tissue stem cell pool. This paracrine secretion of trophic factors is particularly known for MSCs. Local secretion of chemokines and cytokines can stimulate 1) the regeneration of cells by inhibiting apoptosis, 2) neo-angiogenesis, 3) the proliferation and differentiation of tissue-intrinsic stem cells, and 4) cell fusion with transfer of genetic material for cell repair [25].

Evidence in favor of all the above mechanisms may indicate that more than one mechanism contribute to the generation of solid organ-specific cells. Based on our current understanding, among the most likely mechanisms for solid-organ tissue (e.g., cardiac tissue) repair is by differentiation of locally injected hematopoietic tissue-derived progenitor cells into vascular sinusoidal cells resulting in neo-vascularization near the site of tissue injury. This in turn activates the tissue intrinsic stem cell pool (e.g., CSCs) to repair or regenerate solid-organ tissue.

CLINICAL CARDIAC TISSUE REPAIR TRIALS

It should be noted that, up to now, no clinical study has convincingly shown the repair of injured or the generation of new tissue originating from adult hematopoietic tissue-derived stem cells/cells or from solid-organ tissue-intrinsic stem cells. This is explained, among other reasons, by safety issues regarding stem cell marking in an autologous transplant setting and by regulatory issues. Nevertheless, experimental studies held promise of translating tissue repair data to a clinical level.

Over 100 patients in 30 completed or ongoing clinical cardiac tissue repair trials have been studied so far. The outcome of 7 major clinical trials in AMI patients using BM-derived mononuclear cells (6 trials) or skeletal myoblasts (1 trial) shows no significant improvement in left ventricular ejection fraction (LVEF) and significant but still modest improvement in 3 trials [8]. Two meta-analyses published in 2007 confirm those data of modest LVEF improvement in AMI patients only [26, 27]. A most recent randomized clinical trial in patients with re-perfused AMI using MSCs (Prochymal^TM, Osiris Therapeutics, Inc. USA) did not show significant improvement in LVEF at 3 and 6 months post infarction either [28].

The injection of unselected BM- or PB-derived mononuclear cells is a safe therapeutic approach. Re-stenosis

was reported in one trial using CD133-selected stem cells. Clinical trials using myoblasts for tissue repair had an increased risk of arrhythmia probably due to incompatible 'wiring'. Under experimental conditions intramyocardial calcifications and ischemic damage to the myocardium was noticed when using MSCs.

DIRECTIONS AND BASIC QUESTIONS

Gene therapy has been tried in various modifications including transendocardial injection of plasmid encoding VEGF (Euroinject One Trial), adenovirus encoding VEGF, skeletal myoblasts engineered to express the gap-junction protein connexin-43 for enhanced electrical coupling of incorporated cells, and skeletal myoblasts engineered to express VEGF [7, 29].

The field of stem cell-induced tissue repair is still in its infancy. The stem cell or cell population that most likely contributes to tissue repair still needs to be identified. Engineering the stem cell microenvironment is an essential part of tissue repair but still not well understood. Cell delivery systems need to be optimized including MTE technology using cell carrier systems. Finally the stem cell numbers delivered to the site of tissue injury need to be optimized.

More basic considerations address the question of whether stem cell therapy needs to undergo all the developmental steps in cardiogenesis to be effective rather than using more differentiated stem cells that are directed into the cardiac lineage. Does adult stem cell therapy lead to functional and permanent incorporation of newly generated or modified cardiomyocytes? Finally, if paracrine (trophic) factors secreted by transplanted cells and stem cells activate the cardiac resident stem cell pool, could those factors be identified and used directly as a locally injectable drug thus bypassing the cellular mediator?

ABBREVIATIONS

HSCs   =   hematopoietic stem cells

MSCs   =   mesenchymal stem cells

EPCs   =   endothelial progenitor cells

MAPCs =   multipotent adult progenitor cells

CSCs   =   cardiac stem cells

MTE   =   myocardial tissue engineering

BM   =   bone marrow

PB   =   peripheral blood

UCB   =   umbilical cord blood

G-CSF =   granulocyte colony-stimulating factor

AMI   =   acute myocardial infarct

LVEF   =   left ventricular ejection fraction

VEGF   =   vascular endothelial growth factor

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