Regeneration of Myocardium - Dawn of a New Era!

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Abstract

The notion of repairing or regenerating lost myocardium via cell based therapies is highly appealing. The identification of adult bone marrow stem cells and their supportive pre-clinical data fueled the interest in utilizing these cells for physiological relevant cardiomyogenesis. Enthusiasm for cardiac regeneration via cell therapy has further increased by many encouraging reports in both animal and human studies. Further intensive research in basic science paralleled with clinical trials may make cardiovascular regenerative medicine a reality in fighting against congestive heart failure, the leading cause of morbidity and mortality associated with loss of functional cardiomyocytes. Here we review the preclinical phase to clinical phase of stem cell therapy for myocardium and provide a brief overview on unresolved issue and mechanistic insight of the repair.

Introduction

The innovative thinking has always been challenged by existing dogmas and subjected to great hardships. The history of science far from being smooth has faced great many hurdles, as in other fields. The birth of modern science was ushered in by the great intellectual revolution that occurred during the Renaissance when irreverent thinkers began to question centuries-old dogmas imposed on the population. This process continued later and continues till today when insuppressible drives of free minds formulate new concepts and bring about revolutionary changes. Old dogmas are crumbling while new ideas are unfolding.

A veritable revolution is now unfolding in the field of cardiac biology where a fundamental principle that is “the heart is a post-mitotic organ incapable of regeneration” has come under attack of late. According to this dogma, the number of cardiomyocytes we are born with is all we will have for the rest of our lives. If myocytes die they cannot be replaced. This bleak doctrine has been challenged by the hypothesis that adult stem cells which have been used to restore blood and immune system can also regenerate myocytes. Moreover experimental evidences such as regenerative potential of native human myocardium, existence of cardiac stem cells, evidences of endogenous cell recruitment form the systemic circulation within the myocardium and evidences that not only exogenously administered cell population home to the injured heart but also they can reconstitute infarcted myocardium, has compelled us to think otherwise.

This is the need to fight against the current silent killer, the congestive heart failure following myocardial infarction wherein there is reduced cardiac reserve relating predominantly to an early and extensive loss of functioning cardiomyocytes. Congestive heart failure is the common cause of frequent hospitalization after myocardial infarction with 50% of patients dying within 5 years of the diagnosis.

Clinical Need

The pathophysiology of acute myocardial infarction is very complex from the start to the actual attack and its subsequent events. The event sets in, in most of the cases with sudden occlusion of the coronary artery with the formation of thrombus on ruptured plaque. This sudden stoppage of blood supply leads to the process of myocardial necrosis and loss of systolic function. As a compensatory mechanism, heart dilates to maintain the stroke volume- process of adverse remodeling of left ventricle. If this process is not arrested, wall stress increases leading to further dilatation, loss of systolic function, reduced pumping capacity of the heart, congestive cardiac failure and finally death.

Cellular proliferation has been theoretically restricted to endothelial and fibroblast cell populations leading to the development of collateral circulation and scar formation respectively. Cardiac myocytes traditionally considered post-mitotic end-differentiated cells lack the ability of cell division and proliferation following ischemic injury. It however undergoes “hypertrophy” as sole mechanism of compensation for loss of functional myocardium. They contribute to the increase in muscle mass of the myocardium after myocardial infarction. However, their capacity for regeneration, mitigation of the adverse effects of ventricular remodeling and contribution to cardiac function is limited.

On the other end after myocardial infarction, the newly formed capillary network in the infarcted myocardium cannot adequately keep up with the tissue growth needed for contractile compensation and cannot meet the higher demands for oxygen and nutrients of the surviving hypertrophied cardiomyocytes and prevent the apoptosis of the hypertrophied cardiomyocytes, leading to further expansion of the infarct and fibrosis of the myocardium. Congestive heart failure after an extensive myocardial infarction may thus occur when compensatory mechanisms are overwhelmed. The loss of cardiomyocytes combined with the absence of an adequate endogenous repair mechanism contributes towards progression of heart failure. Heart muscle salvage after heart attack is the single important determinant factor for event free long term survival.

According to the historical experiments carried out by Reimer and Jenigs who ligated the coronary artery in a canine model...
and produced an infarction which led to wave front of necrosis starting from endocardium to epicardium. If this ligation is removed and blood is allowed to reperfuse the myocardium, there is a salvage of muscle tissue in a reverse manner starting from epicardium to endocardium in a timely manner. This classic experimental study gave birth to reperfusing strategies. The treatment therefore aimed at restoration of the blood supply by reopening the artery that can be achieved pharmacologically by using fibrinolytic agents\(^\text{6-13}\) which can be delivered at all times by all medical personnel- as well as mechanically by way of removing the occlusion by Percutaneous Transluminal Coronary Angioplasty (PTCA)\(^\text{14-16}\) with balloon catheter within few hours of heart attack. Both these modalities are very effective in reperfusing the myocardium. However the salvage of myocardium is only 2-4% on long term follow-up. After the introduction of these modalities of the reperfusion almost 20 years ago, there has been no further impact on salvage of muscle by all our current treatment modalities. Numerous pharmacological measures exploring various hypotheses have been tried out but none of them were successful in humans. Cardiac transplant seem to be an ideal option for a vast number of these patients, but due to lack of donor hearts cannot meet even a partial demand of it. Other measures like heart assist devices these patients, but due to lack of donor hearts cannot meet even a partial demand of it. Other measures like heart assist devices have been tried out but none of them were successful in humans. Cardiac transplant seem to be an ideal option for a vast number of these patients, but due to lack of donor hearts cannot meet even a partial demand of it. Other measures like heart assist devices and pacemakers have shown not to prolong the survival and are not cost effective. Other modalities like Cool MI, HOT MI, Apex MI, post conditioning of MI, supersaturate O\(_2\) therapy have not held any promise.\(^\text{15-20}\)

Thus in addition to reperfusion therapy we need to regenerate new myocardium. The myocardium consists of three integrated components: myocytes, extra-cellular matrix and the capillary microcirculation that services the contractile unit assembly. Considerations of all these components provide important insights into the remodeling process and a rationale for future therapeutic strategies. Apart from regenerating cardiomyocytes, increasing neoangiogenesis to infarcted myocardium to enhance oxygen and nutrients through the formation of new vessels also has the potential to improve the cardiac function through the rescue of hibernating myocardium and decreased apoptosis of hypertrophied cardiomyocytes. Thus regeneration of both cardiomyocytes and coronary capillaries is what should be focused in improving the viability of myocardium.

Considered as a terminally differentiated organ, regenerating the myocardium was never thought of as an option for heart muscle salvage. Stem cell based therapy became a realistic option to replace damaged heart muscles. Stem cells are the primitive cells; undifferentiated, undefined pluripotent multilineage cells that retain the ability to renew themselves through mitotic cell division and can divide and create a cell more differentiated than it self. Every single cell in the body stems from this type of cell hence the name stem cells. Regenerating myocardium requires differentiation of stem cells into myocardial, endothelial and smooth muscle cells giving rise to myocytes, capillary microcirculation and the extracellular matrix.

It was reported from experimental findings that there was a capability for myocyte turnover in mammalian heart. This and evidence that post natal bone marrow and circulating blood may harbour myocardial and vascular progenitor cells gave a way for preclinical trials. Subsequent promising reports of these same trials prompted rapid initiation of clinical trials.

### Pre-Clinical Data

#### Evidence Towards Regeneration:

- **Evidence of Regenerative Potential of Native Human Myocardium:**
  
  Kajstura et al\(^\text{21}\) in control human hearts by confocal microscopy demonstrated 14x10\(^6\) myocytes in mitosis. These figures were increased nearly tenfold in end stage ischaemic heart disease and idiopathic dilated cardiomyopathy. Thus it was apparent that the slow turnover of myocytes existed providing possible explanation of why pathologies linked with myocyte death such as diabetic cardiomyopathy did not lead to myocardial cellular wipe-out (Cai et al).\(^\text{22}\) In addition, Beltrami et al\(^\text{23}\) by means of labeling for the nuclear antigen Ki-67 in post-mortem infarcted hearts demonstrated 4% of myocyte nuclei undergoing mitosis in the infarct border. The origin of these cells undergoing cellular division could be from a resident cardiac stem cell population or stem cells recruited within the heart from the systemic circulation, ultimately being derived from the bone marrow.

- **Evidence of Existence of Resident Cardiac Stem Cells:**
  
  Hierlihy et al\(^\text{24}\) first described endogenous resident cardiac stem cells in mice. Subsequently Beltrami et al\(^\text{25}\) demonstrated these lineage negative (L-) C kit + (Lin- C-Kit+) cells to be able to differentiate in vitro into all three main myocardial cell types-myocardial, endothelial, smooth muscle cell types. In 2006 Lugwitz et al\(^\text{26}\) using Cre/lox technology were able to specifically mark a resident population of cardiogenic precursor cells in rats, mice and human beings that express islet-1 gene: a gene initially iterated in early embryonic mesodermal cells that were clearly committed to a myocardial lineage.

- **Evidence for Endogenous Cell Recruitment from the Systemic Circulation within the Myocardium:**

  It has been known for many decades that myocardial infarction is an inflammatory disease, which results first in the homing of neutrophils and later monocytes from the circulation (Malloy et al, 1939).\(^\text{27}\) It has been appreciated only recently however, that marrow-derived progenitor cells circulate and home to injured tissues similarly to leukocytes where they contribute to the formation of new tissues (Jackson et al, and Bittiera et al.\(^\text{28, 29}\)). In an interesting experiment by Laflamme et al\(^\text{30}\) and Quaini et al\(^\text{31}\) of sex-mismatched cardiac transplantations, homing of recipient's progenitor cells in the myocardium was demonstrated. In the procedure Y chromosome in-situ hybridization were used to track the male cells in the female allografts coupled with immunostaining to define the identity these cells had acquired. Quanni et al\(^\text{32}\) further hypothesized that the donor hearts harbour a population of resident primitive cells and also attracted a second population of progenitor cells from the recipients. Similar interpretation could be derived from experiment of Bayes-Genis et al\(^\text{33}\) who reported the presence of cardiac chimerism in recipients of peripheral bone and bone marrow stem cells with the use of a sensitive polymerase chain reaction assay for donor and recipient genotyping. Evidence that exogenously administered cell population home to the injured heart:

In addition to tracking endogenous cells, several exogenous administered cell populations have been shown to home to the injured heart after intravenous injection. Kocher et al\(^\text{34}\) and Aicher et al\(^\text{35}\) demonstrated endothelial progenitor cells isolated from blood or marrow to harbour in myocardium. Beltrami et al\(^\text{36}\) demonstrated that when Lin-c-+ kit cells from myocardium
were injected into an ischemic heart, these cells or their clonal progeny reconstitute well-differentiated myocardium, formed by blood-carrying new vessels and myocytes with the characteristics of young cells, encompassing approximately 70% of the ventricle. Oh et al demonstrated that given intravenously after ischemia/reperfusion, cardiac stem cells with stem cell-1 (Sca 1) antigen home to injured myocardium. Similar observation was also reported by Bittiera et al and Ma et al for mesenchymal stem cells isolated from the marrow.

Homing of Stem Cells to Injured Myocardium:

Immediately after an acute myocardial infarction, an intense inflammatory cascade is unleashed. This results in stem cells migration to the site of injury. This is called Homing. At least three major compartments can be thought of to regulate this complicated orchestra, the injured myocardium, the bone marrow, and the peripheral circulation. The injured myocardium is responsible for releasing the signals via peripheral blood to signal the mobilization of the extra-cardiac stem cells from the major reservoir, bone marrow, into peripheral circulation. Following mobilization, these circulating bone marrow-derived stem cells are then able to follow a trail marked by specific signals, subsequently exit the circulation, and home to injured sites to initiate the cardiac repair process (Figure 1). These three players involved in mobilization and homing process must work together to achieve functional significant stem cell-mediated repair and regeneration.

The ability of injured myocardium to recruit extra-cardiac stem cells following injury is critical to aid in myocardial repair and regeneration. Little is known with regard to the regulatory mechanisms that control the homing and holding of stem cells to injured tissues. The precise time course, kinetics and factors stimulating bone marrow mobilization remain the subject of intense investigation; nonetheless, several crucial factors have been shown to promote the mobilization of bone marrow-derived stem cells into peripheral circulation, including granulocyte colony-stimulating factor (G-CSF), granulocyte/macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and erythropoietin (EPO). Myocardial ischemia is known to induce several classically ‘mobilizing cytokines’, including, but not limited to, G-CSF, SCF, VEGF, stromal derived factor (SDF-1), and EPO and these cytokines may be responsible for the observed homing of bone marrow-derived stem cells following MI. Mobilization of endothelial progenitor cells (EPCs) through cytokine stimuli increases EPC concentration in the peripheral circulation substantially.

In addition to well-recognized hematopoietic stem cells (HSCs) mobilizing agents such as G-CSF and SCF, VEGF, and EPO, statins have been shown to promote endothelial progenitor cell recruitment.

G-CSF and SCF/c-kit

SCF, also known as Steel factor, is a ligand for c-kit, a receptor expressed on stem cell and tissue progenitor cells, including resident cardiac stem cells. Similar to G-CSF and GM-CSF, SCF is a hematopoietic factor that is well known to regulate proliferation, differentiation and survival of bone marrow derived stem cells.

SDF-1/CXCR4

Of significant clinical importance and relevance are the cytokine SDF-1 and its cognate receptor CXCR4 which have emerged as important players in this regard. It has been demonstrated by Ceradini et al that SDF-1 gene expression is partially controlled by the transcription factor hypoxia inducible factor-1 (HIF-1) translating thus to increased levels in the presence of tissue hypoxia. HIF-1 is also an early transcription factor for VEGF which is also up-regulated in myocardial infarction (Banai et al, Brogi E, Lee SH). To take advantage of these secreted factors cytokines to improve post-MI cardiac repair and regeneration, thorough investigation of the timing of the release and interactions among signaling factors is required. Despite the existence of cardiac stem/progenitor cells, or evidence of recruitment of stem cells from bone marrow at the site of injury, it is well recognized that this endogenous capacity for regeneration is insufficient to mediate repair following severe cardiac injury. It has to be replenished exogenously by infusion of these stem cells to repair the damage.

Animal Experiments

Initial encouraging data of myocardial regeneration from cellular transplantation of bone marrow derived stem cells came from small animal myocardial infarction models that also proved invaluable in assessing various cell populations for potential myocardial regenerative capability.

In 1999 Tomita et al documented transplantation of autologous bone marrow cells to stimulate angiogenesis, in the recipient ischemic myocardium. In their experiments cryoinjuries were created three weeks earlier. Then Brdu+ cells were transplanted into myocardial scar. These labeled cells were found in hearts examined eight weeks post transplantation. Not only there was increase capillary density in comparison to control groups but these cells were present within the wall of the newly formed vessels. Functional improvement was observed only in recipients of the mesenchymal stem cells that had been treated with 5-azacytidine.

Two years later came the revolutionary paper of Orlic et al with a very provocative finding. They suggested that directly injecting haematopoietic stem cells resulted in extensive myocardial regeneration. These authors took bone marrow from mice expressing enhanced green fluorescent protein (EFGP) depleted the differentiated cells sorted the remainder for expression of stem cell marker c-kit and then injected them into acutely ischemic myocardium after 3-5 hours of

Fig. 1: A schematic representation of cell-based myocardial repair. Signals for mobilization and homing must work in an integrated fashion among the myocardium, peripheral blood, and bone marrow to achieve functionally significant stem cell-mediated repair and regeneration. Adapted from Liao R et al

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coronary ligation. They reported that nine- days after injection regenerative myocardium derived from the donor marrow occupied the majority of the infarct region (left ventricle). The authors concluded that the Lin-c-kit+ bone marrow cells have the capability of regenerating acutely significant amount of contracting myocardium. Further mice receiving stem cells injection showed reduced ventricular dilatation and increased fractional shortening by echocardiography. This article generated tremendous excitement in both the basic and clinical research communities.

At the same time Jackson and colleagues demonstrated that delivered side populations cells or their progeny were able to generate donor myocytes and endothelium. They transplanted an enriched side-population subset of bone marrow hematopoietic cells (CD34-/low C-kit+, Sca+) into lethally irradiated mice then subsequently rendered ischemic by transient coronary ligation.

Kocher et al demonstrated that systemic infusions of human bone marrow-derived endothelial cell precursors were able to interpret the remodeling process of the left ventricle. The observed neovascularization prevented apoptosis of hypertrophied myocytes reducing collagen deposition and subsequent scar formation. Post transplantation ventricle function improved as well.

Orlic et al further hypothesized and demonstrated that mobilization of animal’s own bone marrow with G-CSF before and after myocardial infarction in mice resulted in growth of new cardiomyocytes in the infarct zone, improved ventricular function and substantial decreased mortality by 68%.

In all the above studies cell administration was performed by direct intramyocardial injection. In an attempt to reproduce intraconary delivery two weeks after coronary artery ligation Wang et al injected directly vision mesenchymal stem cells suspension in briefly occluded ascending aortas. Injected cells could be identified after three weeks as cardiomyocytes or fibroblasts. Thus it was concluded that cells migrated through the coronary circulation to sites of injury and under the direct effect of the local microenvironment and transdifferentiated into required phenotypes.

Several more experiments on smaller and larger animal models nearing similarity to humans investigating a variety of cell types in conjunction with various cytokines or even gene therapy technique were initiated and have been published. Fueled in part by hopes for cardiac transdifferentiation as well as by the considerable body of data supporting angiogenic activity, bone marrow studies moved remarkably quickly from small animals to human trials.

Overview of Stem Cells in Myocardial Regeneration:

The most appropriate cell type for restoration of damaged myocardial tissue has not yet been defined. Variable degrees of improvement in cardiac function in models have been observed with transplantation of stem cells from different sources.

1. Embryonic stem cells,
2. Umbilical Cord Blood cells
3. Resident Cardiac stem cells
4. Skeletal Myoblasts
5. Adult Bone Marrow stem cells
   a. Hematopoietic stem cells
   b. Mesenchymal stem cells

c. Endothelial progenitor cells

1. Embryonic Stem Cells:

Embryonic stem (ES) cells are derived from the inner cell mass of the blastocyst-stage embryo, late in the first week after fertilization. They are considered to be totipotent, able to give rise to many different cell lineages. They differentiate into spontaneously beating cells with a cardiomyocyte phenotype. The morphology and ultrastructure of these cells are organized with sarcomeric structure, formation of intercalated disks, desmosomes, and gap junctions, characteristic of cardiomyocytes and they demonstrate the presence of functional syncitium with action potential propagation. When transplanted into infarcted myocardium, ES cells-derived cardiomyocytes engraft and have shown to improve cardiac function in several rodent models. In the failing heart in addition to replenishing cardiomyocytes by ES-derived cells, a simultaneous increase in the blood supply may be necessary for optimal prolonged engraftment. Hence it is of interest that ES cells differentiate to all cell lines necessary for formation of new blood vessels. Both human and murine ES cells spontaneously differentiate to form endothelial and smooth muscle cells in vitro and in vivo. To date no human clinical trials have been initiated because of the possibility of teratoma formation and ethical issues surrounding access to the embryos.

2. Umbilical Cord Blood Stem Cells

In Umbilical cord blood (UCB) stem cells exist in higher numbers than in adult human blood or bone marrow. UCB contains both hematopoietic stem cells and mesenchymal precursor cells for cardiac repair. Ma et al injected human mononuclear UCB cells, a small fraction (≈1%) of which were CD34+, intravenously 1 day after MI in NOD/scid mice. The cells homed to the infarcted hearts, reduced infarct size, and enhanced neovascularization with capillary endothelial cells of both human and mouse origin. Interestingly, they found no evidence of myocytes of human origin, arguing against cardiomyogenic differentiation. In a rat model of MI, UCB CD34+ improved cardiac function when injected into the peri-infarct rim immediately after MI compared with control animals that received injection of medium. Apart from these; Kogler and colleagues have described a population of cells from human UCB called unrestricted somatic stem cells. These cells, which are fibroblast like in appearance, adhere to culture dishes are negative for c-kit, CD34, and CD45 and are capable of differentiating, both in vitro and in vivo, into a variety of tissues, including cardiomyocytes. These stem cells, when delivered by direct injection at thoracotomy in immunosuppressed pigs after MI, improved perfusion and wall motion, reduced infarct scar size, and enhanced global cardiac function. At present, no clinical studies of UCB have been reported.

3. Resident Cardiac Stem Cells:

Resident cardiac stem cells are undifferentiated cells that express the stem cell antigen c-kit, MDR-1 and Sca-1 in variable combinations and capable of differentiating into cardiomyocytes. Since they are cardiac in origin, perhaps such cells might provide a mechanically and electrophysiologically compatible source of cells for transplantation. These cells can be harvested from cardiac biopsies. Injecting these cells in the setting of MI can promote cardiomyocyte function with associated improvement in systolic function (Messina et al). At present there are no
clinical trials under way as these cells are limited in number and require ex-vivo expansion over several weeks.

As far as resident cardiac stem cells are concerned Urbanek et al have demonstrated that cardiac stem cells increase in number immediately after MI, but in the chronic phase, the number falls and the remaining cardiac stem cells have less regenerative potential. This suggests that left ventricular (LV) dysfunction in ischemic cardiomyopathy may be due to defect in or deficiency of functionally competent cardiac stem cells. In addition Mouquet et al have also demonstrated that bone marrow may represent a reservoir for cardiac stem cells and suggests that depletion of this reservoir could contribute to diminished reparative capacity.

4. Skeletal Myoblasts

Also known as satellite cells are a population of progenitor cells which can be isolated from skeletal muscle biopsies. Skeletal myoblasts were the first to enter the clinical arena after completion of a decade of experimental testing resulting in at least 40 studies. These studies consistently showed differentiation of implanted myoblasts into multinucleated myotubes (not cardiomyocytes) but with the absence of electromechanical coupling with host cardiomyocytes. Despite these apparent short comings a definite improvement in regional and global left ventricular functions was demonstrated. These data along with the clinically appealing characteristics of skeletal myoblasts (a high in vitro scalability of the initial biopsy, an advance stage of differentiation virtually eliminating tumorigenicity and a high resistance to ischemia) paved the way for the initial human trials which started in June 2000.

Autologous myoblasts were isolated from muscle biopsies by enzymatic dispersion and the cells were expanded for several weeks in culture by use of fetal bovine serum (tested free from bovine spongiform encephalopathy prion as a mitogen). All studies focused on patients with severe left ventricular dysfunction caused by MI and cell injections were targeted to discrete akinetic and metabolically inactive scars. Four of these studies were surgical i.e. myoblasts were implanted at the time of CABG or at left ventricular assist device implantation and remaining were catheter based procedures and used either an endoventricular or a coronary sinus transvenous approach. Technically there was feasibility of culturing cells and injections into the target area. Long term engraftment of myoblasts aligned parallel to host cardiomyocytes and embedded in scar tissues (up to 18 months).

However 4 out of 10 patients developed serious ventricular arrhythmias soon and required implantable defibrillators. Disorganized myocardial architecture, difference in the activation kinetics of the ion channels between skeletal and cardiac muscle and/or inflammatory responses against the dead myoblasts were coined for the arrhythmogenesis observed in these trials.

No meaningful conclusions could be drawn regarding efficacy in restoration of function in the injected areas. These results indicated that Phase II randomized controlled trials are needed that include common culture processes, variable end points to judge efficacy (stress echocardiography, nuclear angiograms, cardiac magnetic resonance imaging (MRI), positron emission tomography) and the variable baseline functions of the engrafted regions that range from hypokinetic to dyskinetic. Simultaneously further research on skeletal engraftment into cardiomyocyte and improving its myocardial viability is warranted.

5. Adult Bone Marrow Derived Stem Cells:

Bone stem cells gained attention as early as in the year 1968, wherein the first report of their clinical use for restoring the blood and the immune system in children with congenital immunodeficiencies were reported. HSCs were routinely used in clinics, mainly for treating disorders of haematopoietic and immune system. Of particular relevance to the cardiovascular community was hypothesis that bone marrow cells can give rise to new cardiomyocytes.

Adult bone marrow is heterogeneous and contains various stem cell populations. Three distinct cell populations of stem cells reside within the adult bone marrow.

a. CD34+ haematopoietic stem cells (HSCs) -precursors of blood and endothelial cell lineages.

b. CD34- mesenchymal stem cells (MSCs)-precursors of stromal cells including osteogenic, chondrogenic and adipogenic lineages

c. Endothelial progenitor cells (EPCs) or angioblasts. These cells and HSCs are thought to share common precursors.

Apart from these there are Multipotent Adult Progenitors Cells (MAPCs) also derived from bone marrow stromal cells. They have the ability to differentiate in-vitro in cells of three germ layers and differentiate into cardiac, endothelial and smooth muscle cell phenotypes.

It has been recently suggested that the bone marrow harbor additionally non-haematopoietic cells which appear to be highly mobile and express mRNA/proteins for various markers of early tissue committed stem cells. They express markers for cardiac differentiation and it is hypothesized that they are responsible for any observed myocardial regeneration attributed to bone marrow derived stem cells (Kucia et al 2004, 2005).

a. Hematopoietic stem cells (HSC).

HSC can be isolated from bone marrow cells through selective sorting for a particular set of surface (Lineage negative [L-], C-kit+, Sca-1+, CD34+ and CD38+) and represent the prototypic adult stem cell population. Despite the failure of studies to definitely prove differentiation of HSCs into cardiomyocytes, in-vitro, several studies in mice have demonstrated the potential of HSC to differentiate into cardiomyocytes or vascular cells following cardiac injury -in-vivo.

b. Mesenchymal Stem Cells

Within the bone marrow stroma resides a subset of non-hematopoietic cells that have the potential to differentiate into cells of mesenchymal origin. These mesenchymal stem cells (MSC) represent approximately 0.001 to 0.01% of the total nucleated marrow cell population, a concentration 10-fold lower than their hematopoietic counterparts. MSC are self-renewing and expandable in-vitro using standard cell culture techniques. Immunophenotypically, MSC lack the typical hematopoietic antigens (CD45, CD34, and CD14) but express specific adhesion molecules (ALCAM/CAD44 and antigens (SH2/SH3/SH4/STRO-1)). At first, MSC were thought to contribute solely to the formation of the stromal microenvironment in the bone marrow and maintain HSC survival and function. However, subsequent
Cells with phenotypic and functional characteristics similar to the fetal angioblast also are present in adult human bone marrow.61 Endothelial progenitor cells (EPC) represent a subset of hematopoietic stem cells that are able to acquire an endothelial phenotype, in-vitro.120-123. EPC express the hematopoietic stem cell markers CD133, CD34 and the endothelial marker Flk-1 (VEGFR-2).122 EPC can be isolated directly from the bone marrow or from the peripheral circulation and expanded, in-vitro. Kocher A et al61 demonstrated that after MI, intravenously injected EPCs homed to the infarct region within 48 hours.61 At 14 days, there was a marked increase in the number of capillaries in the infarct zone and the peri-infarct rim resulting from the induction of both vasculogenesis and angiogenesis, but there was no change in the non-infarcted regions of the heart. There was a significant reduction in collagen deposition and apoptosis of cardiomyocytes and an improvement in cardiac function on echocardiography.61 It appears that neovascularization induced by these cells leads to the prevention of apoptosis and LV remodeling and may lead to some degree of cardiomyocyte regeneration.124

This evidence that precursors of both cardiomyocytes and endothelial cells exist within the mononuclear cell fraction of adult bone marrow forms the basis for the use of bone marrow mononuclear cells (BMMNCs) in most of the clinical trials (Table 1) to date.

### Human Trials

In just past few years, BMMNC transplantation has become the most widely studied cell-based therapy for human applications (Table 1). Because animal studies demonstrated that tissue damage in MI resulted in bone marrow stem cell homing to the infarcted myocardium61 several trials were carried out using autologous bone marrow stem cells. The first published trial of Strauer et al125 carried out intracoronary delivery of autologous BMMNCs in the infarct-related artery 7 days after MI in 20 patients and had a control group comprising 10 patients who refused the treatment. This method resulted in significantly improved myocardial perfusion and wall motion indexes. In The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI) study;126,127 59 patients after acute MI were randomized to receive either infusion of BMMNCs or ex vivo expanded circulating progenitor cells into the infarct-related artery 4 days after MI and showed improvement in LV ejection fraction from 51% to 58% (P<0.001), as well as significantly enhanced myocardial viability and regional wall motion in the

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<th>Type</th>
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<th>Disadvantages</th>
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<td>Fetal Cardiomyocytes</td>
<td>Cardiomyocyte phenotype</td>
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<td>Skeletal Myoblasts Satellite Cells</td>
<td>Electrophysiologically compatible</td>
<td>Immunosuppression required, Ethical debate, short survival and limited supply,</td>
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<td>Hematopoietic Stem Cells Bone marrow</td>
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<td>Electrophysiologically incompatible- lack of gap junction-arrhythmogenic</td>
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<td>Lack of immunogenicity and autologous transplantation-different lineage of cells</td>
<td>Quantum of cell population not adequate.</td>
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<td>Endothelial Progenitor Cells Bone marrow</td>
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infarct area. However, there was no difference between the 2 active cell treatment groups. In the BOne marrOw transfer to enhance ST-elevation infarct regeneration (BOOST) trial, Wollert and coworkers randomized 60 patients after successful percutaneous coronary intervention for acute MI to receive either intracoronary BMMNCs or standard therapy. They demonstrated an improvement in LV ejection fraction of 6.7% in the treatment group and 0.7% in the control group at 6 months (P=0.0026). In the 18-month follow-up to the BOOST study, the improvement in LV ejection fraction in the cell therapy group was sustained. However, the control group also had improved by this time, and the difference between the 2 groups was no longer significantly different. This catch-up phenomenon in the control group suggests that, rather than the effect of cell therapy being transient, cell therapy may in fact accelerate the postinfarction LV functional recovery that is achievable with standard medical therapy. In an interesting study, Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (RPAIR-AMI) trial, which is the largest clinical cardiac double blind, randomized, placebo controlled trial involving 204 patients showed a significantly higher improvement in angiographically calculated LVEFs (5.5%) compared with placebo injection (3.0%) at 4 months. Subjects were administered autologous bone marrow mononuclear cells suspended in medium (or medium alone as control) through intracoronary infusion 3 to 7 days after undergoing successful PCI. Those with lower baseline EF seemed to benefit more from the cell-therapy and it was associated with a reduction of prespecified clinical end point of death, myocardial infarction and revascularization at 1 year. Fernandez Avilés et al labeled human BMMNCs, seeded on top of cryoinjured mice heart slices, and cultured. BMMNCs and showed the ability of cells to graft into the damaged mouse cardiac tissue. After 1 week, they acquired a cardiomyocyte phenotype and expressed cardiac proteins, including connexin43. In a simultaneous clinical trial 20 patients were transplanted with autologous BMMNSCs to see their effect on postinfarction left ventricular (LV) remodeling. There were no adverse effects on microvascular function or myocardial injury. No major cardiac events occurred up to 11±5 months. At 6 months, magnetic resonance showed a decrease in the end-systolic volume, improvement of regional and global LV function, and increased thickness of the infarcted wall, whereas coronary restenosis was only 15%. No changes were found in a nonrandomized contemporary control group. Thus, they demonstrated that BMMNCs were capable of nesting into the damaged myocardium and acquire a cardiac cell phenotype in vitro as well as safely benefiting ventricular remodeling in vivo.

However, there are contradictory reports as well. Janssens and colleagues did not find any improvement in their primary end point after intracoronary transfer of BMMNCs. However they demonstrated a significant reduction in scar size and an improvement in regional function, but there was no improvement in LV ejection fraction (P=0.36). Their patient population differed from the BOOST trial in that they were reperfused earlier and may therefore have gained only a small benefit from cell therapy because they derived maximal benefit from earlier reperfusion. Another trial by Lunde K et al (ASTAMI) demonstrated no significant LV improvement at 12 month after intracoronary infusion of cells 6 days after MI. The reason given may be the method of cell separation they used.

We in India initiated a trial in June 2005 for treating anterior acute myocardial infarction (AMI) patients with autologous BMMNCs in addition to standard therapy after MI, to demonstrate safety and feasibility of harvesting autologous BMMNCs into the coronary artery and the ability to promote improvement in LV function. Cardiac function analysis of 6 months of 18 patients and ten controls by 2D ECHO, LV angiography and cardiac MRI demonstrated significant rise in EF by 4-8%, significant fall in ESV of 10-25% and marginal fall in EDV as compared to controls who demonstrated rise in EF of 1-3%, and moderate rise in ESV and EDV (Shah et al, 2007). The 24 month follow-up by 2D ECHO sustained the improvement seen at 6 months. Clinical follow-up demonstrated arrhythmias in none although one patient and two controls demonstrated
history of angina, repeated hospitalization was seen in one subject from patients and three from controls. Sonography of Abdomen and Pelvic organ, chest X rays and some routine pathological tests carried out after 24 month of patients who have received bone marrow therapy demonstrated no detrimental long term effects of bone marrow infusion on any organ. This study shows that intracoronary infusion of autologous BMMNCs is safe and feasible after acute MI and shows favourable trend towards improvements of LV function and prevention of ventricular remodeling which determines long-term survival.

Taken together, these studies with control groups suggested that BMMNCs are safe and may improve cardiac function by a substantial and clinically meaningful degree following MI.

In contrast to the acute MI setting, patients with chronic ischemic cardiomyopathy are unlikely to release signals from damaged myocardium to induce stem cell homing. Therefore, an alternative approach to intracoronary infusion of cells in this setting is endomyocardial injection of cells to deliver them to the exact location where their effect is required. Perin et al138 enrolled 14 subjects and 7 control subjects with ischemic cardiomyopathy. The treatment group received endomyocardial injection of ≈30 million BMMNCs. They showed a significant reduction in reversible myocardial perfusion defects and a significant improvement in overall LV function. Notably, they enrolled subjects with significant LV dysfunction at baseline; therefore, their results may be more clinically relevant to the CHF patient group than previous studies that enrolled patients with normal or mildly impaired LV function after MI. Currently, a number of phase II studies are ongoing with BMMNCs.

MSCs also have been studied clinically. Chen and colleagues139 randomized 69 patients after MI to receive intracoronary autologous MSCs or placebo. They demonstrated a significant improvement in global and regional LV function and a significant reduction in the size of the perfusion defect, suggesting that MSC therapy can regenerate infarcted myocardium or protect against LV remodeling. Currently, several studies have been undertaken for allergenic MSCs are in clinical trials for myocardial regeneration in the United States under the sponsorship of Osiris Therapeutics. In addition, the Specialized Centers for Cell-Based Therapy program also plans to conduct clinical trials of MSCs for patients with CHF.

In addition to TOPCARE study that used EPCs, Erb and colleagues140 randomized patients with recanalized, chronically occluded coronary arteries to receive intracoronary progenitor cells or placebo. They mobilized bone marrow cells using granulocyte colony-stimulating factor, harvested them from peripheral blood, expanded them ex vivo, and infused them via the coronary artery. This treatment resulted in significant improvements in coronary flow reserve and cardiac function and a significant reduction in infarct size. Currently, clinical trials of EPC therapy for angiogenesis and myocardial regeneration are in progress that use CD34+ cells from bone marrow that are enriched for EPCs. These cells can be immuno-selected from the mononuclear fraction of bone marrow.

Safety Issues

Although clinical human trials in general have been heterogenous as far as the protocols concerned regarding types of cells, time of delivery, routes of delivery, and most importantly with respect to the end points selected for analyzing the data, they have all demonstrated safety. However there are safety issues which were known to be of concern such as oncogenic transformation, multiorgan seeding, unintended cell differentiation and some such as ventricular arrhythmias and accelerated atherogenesis and coronary thrombosis. Our own trial demonstrated that infusion of these cells did not have any adverse effect on cardiac or extra cardiac organ on long term follow-up of 24 months.137

Unresolved Issues

The ultimate aim of the cellular transplantation remains the regeneration of lost heart muscle along with the reversal of the remodeling process. Thus cellular transplantation should provide all three types of cells; cardiomyocytes, endothelial cells and smooth muscle cells that give rise to myocytes, extracellular matrix and the capillary microcirculation that provide blood supply necessary for optimal and prolonged engraftment. In order to achieve this it should provide enough cells in enough numbers. The crucial points to be considered in the same are 1. What number of cells should be delivered? 2. Which cell populations should be delivered? 3. Which application method is the most efficient? 4. When cells should be transplanted? 5. What should be the clinical end points?

1. What number of cells should be given?

Myocardium contains approximately 20 million cardiomyocytes per gm of tissue.141 The average left ventricle is approximately weighs 200g and therefore contains approximately 4 billion cardiomyocytes. To cause a heart failure an infarct needs to kill approximately 25% of the ventricle (for comparison, infarcting 40% of the ventricle results in acute cardiogenic shock).142 Therefore the myocyte deficit in infarction induced heart failure is in the order of one billion cardiomyocytes. True cardiac regeneration would therefore require restoring approximately one billion cardiomyocytes and ensuring their synchronous contraction via electromechanical junctions with host myocardium.

On the other hand it has also been shown that although most components of myocardium can be derived form extracardiac progenitors; the frequency of repopulation varies widely by cell types.143-145 In a study carried out by Minami et al143 it was demonstrated that endothelial cells are most commonly derived from progenitors averaging 24%. These were followed by peri-neural shwann cells at 11.0% and coronary smooth muscle cells at 3.0%. Cardiomyocytes unfortunately were only derived rarely from circulating progenitor cells averaging 0.04%.

2. Which cell populations should be delivered?

While the ideal cell type for stem cell therapies remains to be determined. To date, bone marrow derived stem cells, isolated from whole bone marrow aspirate, remains the most commonly used cell type for human studies. Unfractionated bone marrow cells gained advantage over above cells due to many reasons. It has the feasibility of procuring, no requirement of in-vitro expansion and above all the availability of mixed population of cells with characteristic for differentiating into various populations of cells. And of course it has no ethical issues.

3. Which application method is the most efficient?

A major goal of cardiac stem cell therapy is to transplant enough cells into the myocardium at the site of injury or infarction to maximize restoration of function. Several different approaches currently are being used to deliver stem cells.
Direct injection into the left ventricular wall has advantage over all methods as one can deliver the cells exactly at the region of interest. Moreover the obstructed blood vessels or poor perfusion often associated with the pathophysiology will be bypassed. The routes of delivery may be via the subendocardium using a percutaneous approach, direct injection into the epicardium at the time of surgery which allows visualization into the area of scar, or via coronary veins.

Direct Injection Into the Ventricular Wall

Direct injection of stem cells is used in patients presenting with established cardiac dysfunction in whom a transvascular approach may not be possible because of total occlusion or poor flow within the vessel of the affected territory. There are 3 different approaches to direct injection.

A transendocardial approach can be used in which a needle catheter is advanced across the aortic valve and positioned against the endocardial surface. Cells can then be injected directly into the left ventricle. Electrophysiological mapping can be used to differentiate sites of viable, ischemic, or scarred myocardium. In a transepicardial approach, cells are injected during open heart surgery. The advantage of this approach is that it allows direct visualization of the myocardium and easier identification of regions of scar and border zones of infarcted tissues. A third approach involves the delivery of cells through one of the cardiac veins directly into the myocardium. The limitation of this approach is that positioning the catheter within a particular coronary vein may be considerably more time consuming and technically challenging.

Transvascular Route- Trans catheter approaches include direct infusion into the coronary arteries/veins which makes it possible to deliver the cells in close proximity to the area of injury. It is well suited to treat patients with acutely infarcted and reperfused myocardium. Intracoronary infusion -The advantage of an infusion is that the cells can be directed to a particular territory. Intravenous approach in the setting of MI is most advantageous in terms of the ease of delivery of cells with minimum of intervention at the acute stage. However the numbers are significantly less with proportionately more cells also found in other organs of the body. Microvascular dysfunction which is common after MI may prove a serious obstacle to the delivery and survival of cells in the infarct zone.

4. When cells should be transplanted?

In the first 48 hours of acute myocardial infarction attack debridement and formation of a fibrin based provisional matrix predominates before a healing phase ensues. At the initial 3-4 days after MI cell adhesion molecule concentration which has not yet declined may promote the transplanted cells into inflammatory process than in the formation of functional myocardium. It is only by 7th day after MI, VEGF concentration peaks and cell adhesion molecule concentration declines. By 2 weeks after scar formation the benefits achieved due to cell transplantation are reduced. Therefore, the ideal time point of transplantation remains 7-14 days.

5. What should be the clinical end points?

Cardiac repair trials need to focus on ventricular function and anatomy as primary end points. Although these measurements are less powerful than morbidity and mortality, the field is not sufficiently advanced to support such outcome-based end points. Because the mechanism through which cell therapy acts is still being characterized, clinical trials that establish mechanistic correlates will be most helpful. For example, studies using magnetic resonance imaging in patients suggest that cell therapy might alter the rate of infarct repair or influence the amount of scar contraction. Positron emission tomography studies have demonstrated increased glucose uptake and enhanced myocardial blood flow in cell-engrafted regions, which provide important information regarding effects on tissue metabolism and perfusion.

Another very useful mechanistic end point for clinical trials is the ability to track cells after they are implanted, for instance, through use of paramagnetic particles visible by magnetic resonance imaging, positron-emitting isotopes, or molecular tracers. Also whenever possible, tissue-based analyses should be included in clinical trial design, either by evaluation of explanted hearts at the time of transplantation or by autopsy of patients who die following cell therapy.

Mechanisms

Stem cells are precursor cells capable of proliferation, self renewal and differentiation into the cardiomyocytes. The genetic and cellular mechanisms that initiate this transdifferentiation are not well understood. One of the concepts is that some form of injury or inflammation is prerequisite for the success of this transdifferentiation.

The microenvironment produced by an acute myocardial infarction is a perfect stimulus for homing and differentiation of the stem cells. This result into increased vascular permeability, expression of adhesion proteins, up-regulation of the homing receptors which is mediated by cell to cell contact and chemoattractants released due to the local injury. The migratory capacity of the stem cells at local sites is highly dependent on VEGF and SDF-1 which are upregulated in the hypoxic conditions.

Once the stem cells have homed, they must now engraft and convert into cardiomyocytes and get connected to the adjacent cardiomyocytes with the formation of protein N-Cadherin and Connexin 43 which are the constituents of the gap junctions. Equal and of paramount importance is increased neovascularisation to keep up with the increased metabolic requirements of the newly transplanted cells. The presence of cytokines, VEGF and other factors have paracrine effect which leads to increased neoangiogenesis and vasculogenesis which supplies the added blood supply and the nutrients to the new cells.

However from all the preclinical and clinical trial outcomes discussed above which could not pin-point a clear fate of the engrafted cell in the injured heart, one common observation in most of these studies was the ability of wide range of non-myogenic cell types (embryonic stem cells, skeletal myoblasts, bone marrow cells and resident cardiac progenitors) to improve ventricular functions. Moreover despite approximately 6000 fold difference in number of cells delivered across a broad range of studies, the scale of improvement of myocardial functions seems somewhat similar in each case.

There are four possibilities put forth for the improvement seen in the myocardial function. Transdifferentiation of bone marrow cells into cardiac myocytes, activation of resident cardiac stem cells by bone marrow derived cytokines, proliferation
of residual viable myocytes induced by bone marrow derived cytokines and fusion between bone marrow cells and surviving myocytes.

The first mechanism, transdifferentiation, however faced experimental evidence against and also it was seen from the animal studies that improvement in left ventricular function occurred within 72 hours, which is way too soon to be explained by the occurrence of regeneration as a result of donor cell transplantation. Also it is implausible that the fusion between a bone marrow cell and a preexisting myocyte will result in improved function as there is no evidence or theoretical reason to support this conjecture. The second and third option seemed most acceptable wherein paracrine effects of delivered cells on threatened native myocardial and vascular tissue in the setting of acute injury may not regenerate into myocardium but provide signaling molecules that may lead to activation of resident cardiac progenitors, cytokine induced proliferation of residual cardiomyocytes increased cell survival, protection or regeneration of myocytes, or the promotion of angiogenesis and reduced apoptosis.

Future Directions:

The amount of myocardium salvage in various human trials is not sufficient and encouraging with the present technology of harvesting the cells and implantation. This should propel new technologies and different means to enhance the bioavailability of cells and retention of cells in myocardium.

In both animal and human experiments research; mesenchymal stem cells have emerged as most promising cell population with their inherent property of transdifferentiating into cardiomyocytes and also to be tolerated by the immune system giving us the most convenient “off the shelf” reagent. Umbilical cord blood cells are the richest source of mesenchymal cells which can be used within the strict constraints of ethical guidelines. At Diamond Jubilee celebration of Government Medical college of Nagpur, Dr. Lele has proposed the establishment of Donor Umbilical Cord Blood Cells where 13,000 delivery take place annually under one roof (personal communication). These could be a source of autologous transplantation wherein combination of 3-4 cord blood cells can be combined. This proposal has been accepted by the government of Maharashtra and has opened the door for autologous transplants of various purposes. Such support from governmental organizations or charities will be required to ensure that cell therapies, which may be efficacious but commercially less attractive (e.g., unselected Bone Marrow Cells), will undergo much-needed further clinical testing. As techniques to improve engraftments and bioavailability were being developed, investigators returned to the laboratory to develop better models. NOGA has emerged as one of the technique for transplanting the cells directly into the myocardium by electromechanical mapping during trandsendocardial approach to discriminate between the ischemic and viable regions and for targeted delivery of cells. For imaging of these cells further one will have to optimize on the labeling isotope that will not be compromising on the efficiency of cells to regenerate the myocardium. At present labeling strategies for in vivo surveillance and tracking of various cell populations are super-paramagnetic iron oxide (SPIO) in MRI, Indium-111 (In) Oxin, Technetium (Tc), and fluorodeoxyglucose (= F18-FDG) for direct labeling of cells by radionuclide imaging and PET scanning. Additionally genetic labeling with reported genes that can be traced with imaging probes have been introduced, that will allow for repeatable tracking of cellular and subcellular function over a long period of time. Further pharmacological and genetic strategies that seem to offer enhancement of stem cell retention, engraftment, differentiation and paracrine capability, deserves further exploration.

Summary

Acute myocardial infarction as the end point of complicated atherosclerotic path or thrombotic event is a major cause of world wide morbidity and mortality. The loss of viable myocardium during AMI serves as a main predictor for contractile ventricular dysfunction, the occurrence of acute complications and

**Fig. 2 : Proposed Mechanism of Myocardial Repair**

STEM CELL THERAPY

(BMCs, EPCs, FCs, SMCs, MCs)

SECRETION OF GROWTH PEOTROPIC FACTORS OR CELL SIGNALING PEPTIDES

- DECREASED COLLAGEN EXPRESSION
- ANGIOGENESIS
- INHIBIT APOPTOSIS
- MYOCYTE REGENERATION

DECREASED INFARCT EXPANSION

MYOCARDIAL PRESERVATION

ATTENUATION/ INHIBITION OF POST MI REMODELING

BMCs-Bone marrow stem cells, EPC-Endothelial Progenitor Cells, FC-Fetal Cardiomyocytes, SMC-Smooth Muscle cells, MCs-Mesenchymal cells
The possibilities of using stem cell based therapies for people suffering an AMI have captured the imagination of both the medical and popular communities. Since early reports in animal models more than ten years ago, the stem cell field has made enormous advances in moving towards clinically applicable treatment options and we now stand at the dawn of a new era. This is possible in the field of regenerative medicine with parallel conduct of animal studies and clinical trials. Many times science profits from the clinical trials which raise the basic questions which molecular scientist can work on and answer them. Thus, the argument that these clinical trials should be delayed until mechanisms are further understood will unnecessarily deprive a large number of patients of a new therapeutic approach that may improve their clinical outcomes. Another compelling argument for moving from bench to bedside is that the results of these clinical trials often provide pivotal insights that allow a new field to advance. Several examples in cardiovascular medicine can explain this concept. The fibrinolytic therapy by various trial and errors in clinical experiments provided the basis for planning the first prospective randomized GISSI trial which then clearly demonstrated the benefit of the fibrinolytic therapy in salvaging the myocardium. Similarly ACE inhibitors and HMG-Co Reductase inhibitors, widely used drugs today with solid mechanistic underpinning for their use were both appreciated after entry into clinical practice to have pharmacological effects that extended their use beyond their initially intended design. The additional mechanistic effects were suggested by results obtained in clinical trials demonstrating their importance of synergy between the animal studies and clinical trials conducted in parallel. Another important development of the last decade which totally opened a new therapeutic concept and a separate sub-specialty in cardiovascular medicine is percutaneous transluminal coronary angioplasty without adequate pre-clinical experimental and clinical data. Today primary angioplasty has become the treatment of choice for AMI and has revolutionized the care of cardiac patient. This would not have been possible had Andreas Gruntzig not ventured into clinical trials immediately after doing experimental trials in eight dogs and human postmortems. He immediately published the first series of five patients and the first study of 50 patients followed the next year. This translational research program introduced PTCA as a new strategy but was not free of serious complications. Soon the problems of acute and sub acute thrombosis, acute closure and then the problems of restenosis after stent implantation were appreciated. This prompted research and development of new drugs like Clopidogrel, high-pressure dilatation and drug eluting stents to counter the problems that were introduced. The safety and feasibility of these new trials of drug eluting stents have been in less than 100 patients, much less than the number of patients enrolled in stem cell trials. Thus step-wise progression to clinical trials was appropriate and timely. We anticipate a similar iterative pathway for development of stem cell therapy. Many treatment options and data in animal models did not translate into benefits in human trials. A number of reasons may account for disparity between the pre-clinical and clinical results. The most important difference between the two situations is the complex biology and the multitude of factors in clinical conditions that can never be replicated in experimental models. Thus at some point in the discovery of any treatment modality, only translation into clinical trials will provide necessary data to move forward. There is also a need for ongoing basic research in parallel to advance stem cell therapy towards clinical applications.

Conclusions

At present there is not enough data to implement stem cell therapy as a guideline for the management of AMI. However there is a wealth of pre-clinical and early clinical data showing immediate and long term safety, feasibility and early efficacy of adult cell based therapies. Thus adult cell based therapies should now progress into randomized, placebo controlled double-blind clinical trials.

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B O O K  R E V I E W

Tuberculosis
2nd Edition

Tuberculosis, the second edition, by Dr. S.K. Sharma and Dr. Alladi Mohan is an excellent compilation of available knowledge on TB.

The first edition that came about 7 years ago had all the relevant information of that time. In 7 years science has advanced by leap and bound. It is pertinent that they have brought out the second edition.

The updated second edition is thoroughly and extensively revised and the experiences of a lot of experts from India and all over the world are incorporated. It represents the global perspective of TB. The new pathophysiological process of development of disease, the immuno-molecular level understanding, newer drugs, newer vaccines, newer tests and newer national and international strategies are very well incorporated in the book.

Second edition is more elaborate, colorful with lots of pictures and radiographs, has a lot of new topics on advances that took place in last decade with additional new topics. Public health aspects of TB are very well covered in great detail which would interest policy makers for sure.

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Dr. Agam Vora
Chest Physician