The Use of Bone Marrow-Derived Circulating Progenitor Cells in the Management of Acute Myocardial Infarction

By

Milan Samarage,
Dhaneesha Senaratne,
Charlie Pittaway

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Abstract

Myocardial Infarction is at present the leading cause of mortality and morbidity worldwide. However, Cardiac Stem Cell Therapy is emerging as a revolutionary methodology for treating patients with heart disease. Many advances have been made in the past decade in this field and several clinical non-randomised trials have led to an increased rate of survival. This theoretical study aims to show that myocardial infarction and other related cardiac diseases can be best treated using bone marrow-derived Circulating Progenitor Cells. This paper shall address the pathology of myocardial infarction and will cover possible cellular-based treatments for this disease using Circulating Progenitor Cells and discuss potential complications and ethical considerations.

Introduction

Since the discovery of pluripotent stem cells in adult humans, commonly known as adult stem cells, there has been a significant deviation from Embryonic Stem Cells which are heralded as having the best potential for differentiation, the favourable ethical dimension in the use of non-embryonic stem cells being one of the more potent reasons behind this. The most striking case in point can be drawn from the animal kingdom; where it has been scientifically recorded that the zebrafish (Brachydania rerio or Dania rerio) has the ability to regenerate its heart fully after a 20% left ventricular resection\(^1\). Following from this, there have been considerable trials carried out in both stem cell research as well as gene-therapy in order to duplicate such a regeneration process within the human heart. Innovative work conducted by Orlie et al.\(^2\) using laboratory mice has demonstrated that bone-marrow can be a source of progenitor cells with the ability to differentiate into cardiomyocytes and restore cardiac function following myocardial infarction (MI). Alongside this, research conducted by the Bonn’s University Clinic has produced findings that prove the existence of CXCR4\(^+\) cells that express mRNA/proteins for various markers of early tissue-committed stem cells (TCSCs) that also have the ability to pass through the blood to defective organs and replace diseased tissue\(^3\&4\). It is at present unclear whether there is any substantiation that these collaborated findings are of a singular order of progenitor cells, or if indeed distinct, whether the circulating stem cells too possess the potential for regenerating cardiomyocytes, however, and research is ongoing to understand more of the intricate bio-chemical and genetic workings of these cells. Nevertheless, the fact that progenitor cells are capable of neovascularisation; the result of several processes including angiogenesis, arteriogenesis and possibly vasculogenesis as defined in the study by David L et al.\(^5\), and the fact that the CXCR4\(^+\) class of progenitor cells are capable of being translocated through the circulatory system presents immense opportunities for developing effective non-invasive treatments for heart disease, in particular, myocardial infarction. This theoretical review explores the basic pathophysiology of myocardial infarction as well as possible mechanisms by which circulating stem cells can be applied in repairing the heart damaged by ischemic injury.
Discussion

Basics of Myocardial Infarction

Cardiovascular disease is the leading cause of mortality and morbidity worldwide. The main supply of oxygen to the heart muscle is via the coronary arteries. When insufficient oxygen reaches the heart muscle, or myocardium, ischemia occurs. Myocardial ischemia can occur if the lumen of a coronary artery is narrowed by an athermanous plaque or if the plaque fissures and becomes the site of a thrombus formation which would thus obscure the flow of blood through the vessel.

Myocardial ischemia can also occur from other forms of diseases, such as cardiovascular syphilis and rheumatic heart disease. The general term coronary heart disease or CHD is used in reference to cardiac disease specifically due to atherosclerosis of the coronary arteries. Myocardial ischemia can give rise to the condition angina pectoris. Symptoms of this condition include a dull discomfort in the substernal area and pain in the upper epigastrium which may radiate to the neck and down one or both arms.

Myocardial infarction, commonly known as a heart attack, occurs when prolonged ischemia leads to necrosis or cellular death of heart muscle cells. Confirmation of a diagnosis can be made by characteristic electrocardiogram traces and changes in serum enzyme levels. If left untreated these necrotic sites significantly increase the probability of thrombi reforming due to the destruction of the intimal layer of the coronary vasculature. Apart from this, other lesion prone sites are formed where more advance lesions are frequently found, caused by increased residence time of lipoproteins and macrophage foam cells at the endothelial surface. This in turn is caused by many factors the best known of which is a high level of plasma cholesterol (Low Density Lipoprotein - LDL).

When an atherosclerotic lesion ruptures the sub-endothelial layer is exposed to the blood and a series of normal protective biochemical reactions occur in attempt to close the rupture: coagulation occurs, platelets aggregate and a thrombus is formed. If the thrombus occludes the artery then thrombosis occurs, leading to ischemia. In this condition it has been observed that endothelial cells release a variety of enzymes, cytokines and other proteins which produce a range of effects most of which lead to cascading blood clotting and its related autoimmune response. [figure 1]

<table>
<thead>
<tr>
<th>Biochemical effect</th>
<th>Biochemical response</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactions which promote thrombosis</td>
<td>Initiates blood coagulation</td>
<td>Release of this factor signals the initiation of coagulation cascade. Synthesis of this factor is normally repressed</td>
</tr>
<tr>
<td>Promotes synthesis and secretion of tissue factor</td>
<td>Cofactor required for efficient platelet adhesion to extracellular matrix</td>
<td>Released in response to local tissue injury to facilitate formation of haemostatic plug into a thrombus.</td>
</tr>
<tr>
<td>Increased synthesis and secretion of von Willebrand factor</td>
<td>This protein enhances neutrophil adhesion to endothelial cells</td>
<td>Neutrophils may enter intima in atherosclerosis and inflammation</td>
</tr>
<tr>
<td>Increases P selectin expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactions which promote thrombolysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increases synthesis and secretion of tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI)</td>
<td>PA-I is normally in excess, forms a complex with tPA and the complex is cleared from the blood. tPA stimulates the conversion of plasminogen to plasmin, the enzyme which hydrolyses fibrin.</td>
<td>tPA promotes thrombolysis</td>
</tr>
<tr>
<td>Increased synthesis and secretion of prostaglandin</td>
<td>Inhibits platelet aggregation, promotes vasodilatation</td>
<td>Limits growth of intravascular thrombus</td>
</tr>
<tr>
<td>Increased synthesis and secretion of nitric oxide</td>
<td>Inhibits platelet aggregation, promotes vasodilatation</td>
<td>Limits growth of intravascular thrombus</td>
</tr>
<tr>
<td>Reaction with antithrombin on endothelial membrane</td>
<td>The antithrombin-thrombin complex dissociates and is cleaved from the blood by the liver</td>
<td>May act synergistically with prostaglandin in vivo.</td>
</tr>
<tr>
<td>Reaction with thrombomodulin</td>
<td>Properties of thrombin change when bound to endothelial surface-thrombomodulin</td>
<td>Reduction in active thrombin</td>
</tr>
</tbody>
</table>

[figure 1]
Stem Cells for Myogenesis

Preliminary, uncontrolled, human studies have shown that circulating progenitor cells (CPCs) had successfully incorporated into cardiac scar tissue and thus had reduced remodelling and left ventricular dilation\(^6\). The precise mechanism of this reduction in scar tissue caused by ischemia is currently unknown. However, in another non-randomised test it has been proven that the number of CPCs increases in response to local tissue ischemia\(^7\). This process occurs in reaction on specific hormones such as GM-CSF. The progenitor cells home to and incorporate into ischemic areas where they differentiate into and regenerate cardiomyocytes. Despite these remarkable experimental observations there is, unfortunately, to date no exclusive surface marker for these CPCs. However a few clinical trials have yielded positive results and reaffirm the potential of these stem cells for therapeutic use. The most distinguished clinical trial, the TOPCARE-AMI (transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction) trial yielded conclusive results that Bone marrow-derived progenitor cells (BMPCs) with circulating ability showed significant increases in Left Ventricular ejection fraction (LVEF) and an overall improvement in Left Ventricular dimensions compared with historical controls\(^8\). Further related trials showed that this procedure led to engraftment of 14-39% of the cells which were however, injected intravenously. A noteworthy feature of these trials is that the patients treated were subjected to BMPC-mobilising cytokine called G-CSF before the procedure. It has as of yet not been fully clarified as to what the precise mechanism is of the human body producing this cytokine, though another similar study into circulating stem cells has revealed that the body has a natural process whereby BMPCs were made to transdifferentiate into nerve cells in the brain. Therefore it can be theorised that such a natural mechanism may indeed exist for myogenesis. However, it is uncertain whether such a mechanism solely would be able to regenerate cardiac tissue completely. Thus it is worthwhile researching into a potential therapeutic procedure whereby BMPCs can be utilised to strengthen or entirely replace the natural myogenetic process described above.

Theoretical Mechanism

A theoretical mechanism could work on the following guiding principles based on current scientific findings. However, there are certain limitations which will be discussed later on. The main aims of this procedure shall be to treat post myocardial infarction, i.e. infarcted cardiac tissue using a cellular therapy with minimum if not no invasive procedures, and to restore Left Ventricular ejection fraction (LVEF) and Left Ventricular dimensions to pre-myocardial infarction figures.

The mechanism would involve;

- **Mobilisation** of bone marrow-derived progenitor cells (BMPCs) being mobilised from the extracellular matrix of the bone marrow stroma to the ischemic region of the myocardium. This can be achieved using many cytokines. According to current findings, the most preferred cytokine which has been proved to be effective is the G-CSF cytokine (Granulocyte colony-stimulating factor). Patients in the front-integrated revascularisation and stem cell liberation in evolving acute myocardial infarction by granulocyte colony-stimulating factor (FIRSTLINE-AMI) trial all of whom had undergone primary percutaneous coronary intervention were randomised to receive 10μg/kg of G-CSF for six days in combination with conventional treatment\(^9\). Other known mobilisation factors include certain haemopoietic growth factors, and other
protein factors mentioned in Table 1, though they are rarely selected for the purpose at hand due to their broad speciality and their complication with the accumulation and synthesis of lipoproteins in the blood which in fact the on setting cause of thrombi and sub sequential myocardial infarction. Thus it can be seen that initial step of mobilising the BMPCs is indeed viably feasible and can result in an adequate mobilisation of progenitor cells to the affected areas of the myocardium.

- **Growth** and **Amplification** of progenitor cells should be a concurrent step in the mechanism together with mobilisation. Clinical research is currently underway using erythropoietin amongst many other haemopoietic growth factors in an attempt to use these existing growth factors or to artificially synthesise chemically similar factors to induce progenitor cells to divide. Another protein enzyme called the Mitosis-promoting factor (MPF) or M-phase promoting factor has been identified in embryology studies, and if the genetic mark-up of the synthesis of this protein can be deciphered, it could prove to be yet another important factor in encouraging amplification of progenitor cells. However, in order to minimise risks of a malignant condition from arising the progression of amplification must be closely regulated with an inhibiting enzyme or a form of tumour necrosis factor. Current research on the observation of progenitor cells differentiating into brain cells ought to provide valuable insight as to the best bio-chemical pathway to employ in this instance.

- **Chemoattraction** of the progenitor cells on to the infarcted tissue is a vital step in the mechanism and is unfortunately one of the most poorly understood areas in this field of research. Presently it is been realised that a cytokine, Stromal-derived factor 1 (SDF-1) is one of the crucial chemoattractants for recruiting BMPCs in ischemic cardiomyopathy and is consistent with many published research projects\(^\text{10}\) [figure 2].

- **Differentiation** and **proliferation** of chemoattracted BMPCs into cardiomyocytes would be next logical step and involves many complicating processes only somewhat understood at present. HGF has recently shown to increase both proliferation and survival of cardiomyocytes by directly affecting GATA-4 expression\(^\text{11}\). Another cytokine factor known as LIF has also been reported to enhance survival of cardiomyocytes and to induce regeneration of the myocardium after myocardial

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**figure 2**

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infarction. Thus it can be seen that through current erudition it can be seen that the HGF factor plays an important part in the expression of the GATA-4 transcription factor which is directly linked with the differentiating process of BMPCs into cardiomyocytes.

- Regulation of the whole mechanism is by far the most problematic step and is greatly affected by the many scientific limitations. However, the aim should be to form a manner of regulatory biochemical process that is dependant on the supernatants discharged by infarcted myocardium. For instance, BMPCs can be genetically modified to express certain receptor proteins (currently unknown) that would be amenable to these supernatants would ensure that differentiation of BMPCs would occur proportionately according to the level of infarction.

Therefore, it be seen that a proposed mechanism such as this is indeed feasible in relative terms and would offer an revolutionary new way of treating myocardial infarction, and perhaps more importantly would have long lasting effects in the prevention of further necroses in post-myocardial infarction tissue and as mentioned earlier, may in fact result in a reassertion of cardiomyocytes into scar tissue, with the direct result of improving Left ventricular ejection fraction. (LVEF)

Conclusion

There are, unfortunately, quite a few main problems likely to be encountered in this pilot research project. Firstly, despite hormonal or proteinic therapies, the number of potential cardiac progenitors residing in the bone marrow stroma initially may be too low and thus prove insufficient to reconstitute the myocardium following extensive cardiomyocitic loss brought upon as a result of myocardial infarction. Secondly, there is at present no definite methodology, short of conducting a randomised Phase III clinical trial, of ascertaining the viability of BMPCs repair and regenerate at a faster rate than the generation of scar tissue without the intervention of growth factors and amplifying cytokines which would need to be thoroughly modelled before induction. Another added complication would be the likely competition the BMPCs would have to face as other CXCR4+ circulating stem cells may express genes and differentiate into macrophages or lymphocytes as they too would share the same ‘niche’ and could pose a threat that would compound the infarctious condition. Finally it has been established that BMPCs are age dependent, i.e. they tend to lose their plasticity as well as their regenerative ability with age due to processes not yet fully understood, and could thus be a limitation since myocardial infarction itself is dependent on age as being a risk factor.

Despite these limitations, BMPCs remain a valuable resource in the search for more effective cellular treatments for myocardial infarction. All ethical controversies with regard to Embryonic Stem Cells (ESCs) which society must face are to a great extent made defunct owing to the autologous nature of these BMPCs and the only remaining consideration is that of safety and methods of conception of this approach, which can inevitably be remedied through a sound scientific principles and methodologies in time.

Based on the proposed mechanism as mentioned earlier, it can be promulgated that a strategic combination of the best growth factors, cytokines or genes coupled together with
cellular therapies as outlined above, may lead to a successful new way of dealing with cardiovascular disease.

The development of therapeutic modalities using stem cells for patients with cardiovascular disease is certainly one of the crucial tasks for medicinal researchers. And at present though we have a relatively high understanding of the intricate workings of these fascinating cells within our body, we are still constrained by our lack of clear definite understanding of the genetics and biochemistry of stem cells. Despite this, the non-randomised Phase I clinical trials already being conducted have unveiled a vast amount of hitherto unknown facts surrounding stem cell research and would undoubtedly be of great importance in future investigations. In conclusion, it can be seen from this brief abstract that Bone marrow-derived circulating Progenitor Cells/Stem Cells (BMPCs) possess great potential for the development of valuable novel therapies through international scientific co-operation for the restoration of cardiac function.
References


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