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Review article

Oxygen cycling to improve survival of stem cells for myocardial repair: A review

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ABSTRACT

Heart disease represents the leading cause of death among Americans. There is currently no clinical treatment to regenerate viable myocardium following myocardial infarction, and patients may suffer progressive deterioration and decreased myocardial function from the effects of remodeling of the necrotic myocardium. New therapeutic strategies hold promise for patients who suffer from ischemic heart disease by directly addressing the restoration of functional myocardium following death of cardiomyocytes. Therapeutic stem cell transplantation has shown modest benefit in clinical human trials with decreased fibrosis and increased functional myocardium. Moreover, autologous transplantation holds the potential to implement these therapies while avoiding the immunomodulation concerns of heart transplantation. Despite these benefits, stem cell therapy has been characterized by poor survival and low engraftment of injected stem cells. The hypoxic tissue environment of the ischemic/infracting myocardium impedes stem cell survival and engraftment in myocardial tissue.

Hypoxic preconditioning has been suggested as a viable strategy to increase hypoxic tolerance of stem cells. A number of *in vivo* and *in vitro* studies have demonstrated improved stem cell viability by altering stem cell secretion of protein signals and up-regulation of numerous paracrine signaling pathways that affect inflammatory, survival, and angiogenic signaling pathways. This review will discuss both the mechanisms of hypoxic preconditioning as well as the effects of hypoxic preconditioning in different cell and animal models, examining the pitfalls in current research and the next steps into potentially implementing this methodology in clinical research trials.

Contents

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1. Introduction

Heart disease currently kills about 600,000 Americans each year, and represents the leading cause of death in the United States [1]. In a statement from the American Heart Association, the prevalence of cardiovascular disease (CVD) is expected to increase by approximately 10% within the next two decades with concomitant increases in healthcare associated costs [1]. Myocardial infarction (MI) comprises over 20% of CV deaths [2]. In one European study, the five year survival rate following an acute myocardial infarction (AMI) was only 55% [3]. In part, as a consequence of successful treatment options following MI, the incidence of heart failure is expected to rise over the next decades. Currently, heart failure kills over 300,000 people each year [4]. As cardiac function declines in the setting of heart failure, the only viable option to significantly improve cardiac function is heart transplant, a therapy limited by the shortage of donors [5]. Thus, effective treatment methods in the post-MI patient to prevent subsequent development of heart failure are lacking. The need for better treatment options continues to grow with an aging population and an increasing incidence of heart failure.

Current standards of therapy for AMI include thrombolysis and percutaneous coronary intervention (PCI). These treatments [6], though effective in restoring blood flow to the heart and preventing further damage caused by ischemia, are limited in that they cannot regenerate the damaged tissue following occlusion of coronary vessels. Following damage, the body can do little to repair the weakened myocardium. The potential neocardiomyogenesis, following adverse cardiac events is low [7,8]. As a result, research has been devoted to finding alternative methods to restore viable cardiac tissue within the damaged heart. Since the initial use of stem cells to treat heart failure in 1998, the number of preclinical and clinical trials has increased dramatically. The first study of stem cells following AMI occurred in 2001 with bone marrow derived cells, showing improved myocardial function and cardiac regeneration nine days after MI [9]. Subsequently, other types of stem cells have been studied in an attempt to maximize cardiac regeneration.

2. Clinical and preclinical studies of stem cells therapy for treating MI

In preclinical rodent trials, stem cell therapy following MI, resulted in decreased fibrosis and cell apoptosis and increased contractility [8]. The exact mechanism of this benefit remains incompletely elucidated. Three mechanisms have been predominantly explored; paracrine signaling [10–12], differentiation into cardiac and vascular tissue *de novo* [9], and fusion with pre-existing cardiomyocytes [13]. Additionally, a number of *in vivo* and clinical studies have been attempted. In the Phase 1 CADUCEUS trial, Makkar et al., reported decreased scar mass, increased viable heart mass and improved contractility in 31 patients using autologous cardiosphere-derived cells [14].

Despite research advances, many barriers remain before stem cells can be effectively utilized in the failing or damaged heart. One of the principal impediments to stem cell therapy is the survival of stem cells after transplantation. In one study, Amsalem et al., demonstrated low viability of transplanted stem cells in a rat model, suggesting that the injected cardiomyocytes do not take up residence, or engraft, into heart muscle tissue [15]. In another study, Jackson et al. demonstrated that only 2% of injected stem cells were able to engraft in the infarcted heart [16]. The harsh, ischemic myocardial environment may impede successful survival and engraftment of injected stem cells. However, very little research has been devoted to elucidating whether other factors might play roles in inhibiting stem cell engraftment.

Perhaps due to poor engraftment and/or a host of other factors, no studies have determined long-lasting improvements with stem cell treatments. A long-term clinical study on the efficacy of bone marrow transplantation in patients with MI showed increase in ejection fraction at six, but not 18 months post-treatment [17]. In the CADUCEUS study, no changes in ejection fraction, end-diastolic volume, or end-systolic volume were seen at six months post-treatment [14]. Thus, the use of stem cell therapies following MI have yielded only limited success. Finally, one of the primary benefits of stem cell transplantation is the potential to use autologous cells, removing the need for immune modification treatments post-transplant. If perfected, stem cell transplantation for regeneration of the failing heart has the potential to revolutionize the way these diseases are treated in a clinical setting.

3. Alternative approaches to improve the survival of transplanted stem cells in the ischemic heart

To improve the benefits of stem cell treatment, several alternatives have been explored to increase the efficacy of stem cell treatments in the failing or damaged heart, including stem cell exosome therapy [18–20], drug therapy [21–24], bioengineered delivery systems [25], genetic modification [26], and microRNA therapy [27]. This review focuses on the strategy of hypoxic preconditioning (HP) of stem cells as an adjunctive treatment to improve stem cell therapy in the failing heart (Table 1). The theory behind HP is to increase cell defense mechanisms following exposure to a sub-lethal level of cell stress, ischemia or hypoxia. By conditioning the cells prior to transplant into the ischemic cardiac tissue, increased survivability of progenitor cells is expected, leading to more complete and lasting improvements of cardiac function following MI and heart failure. In addition, secreted factors are expected to benefit injured, viable cells in the ischemic myocardium, promoting survival of penumbral cells and reduce infarct size in the setting of ischemia and ischemia/reperfusion injury [17–19] HP cells exhibit better survival, metabolic patterns, and produce more angiogenic factors [28–34]. HP of stem cells has also led to better heart function in some *in vivo* models.

4. Hypoxic preconditioning, an overview

There are several different types of hypoxic preconditioning under investigation. To further complicate the clinical approach, a variety of cell types have been researched, including mesenchymal stem cells [35], human adipose tissue-derived stem cells [36], cardiospheres [14], bone marrow stem cells [11], cardiac progenitor cells [22], embryonic stem cells [37], mononuclear cells [38], and induced pluripotent stem cell-cardiomyocytes [39].

While not discussed in this review, it is important to note that HP of whole organisms has also shown beneficial effects [40–42]. Some researchers have mimicked long-term hypoxic conditions through the use of hypobaric chambers, exposing animal models to various degrees and durations of hypoxia with observed changes in levels of tissue injury [43]. However, in previous studies stem cells have been exposed to extreme hypoxic conditions for multiple days [36], a strategy not feasible in *in vivo* models due to the severity of hypoxia. Finally, it is prudent to emphasize that the level of hypoxia subjected to different types of stem cells varies significantly between researchers. Anoxic preconditioning has been used in a study to enhance cardioprotection of transplanted stem cells [44], but most HP studies use low oxygen concentration as opposed to complete anoxic conditions (see Fig. 1). The differences between different degrees of hypoxia in HP are largely unexplored.