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A novel high throughput approach to screen for cardiac arrhythmic events following stem cell treatment

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Abstract

Although stem cell therapy is promising for repairing damaged cardiac tissue and improving heart function, there are safety concerns, especially regarding the risk of arrhythmias, which can be life threatening. To address this issue, we propose to develop a novel screening system to evaluate arrhythmic risk associated with stem cell therapy using a high-throughput multielectrode array system that can measure conduction velocity and action potential duration in cardiomyocytes co-cultured with different types of stem cells, such as mesenchymal stem cells, skeletal myoblasts, and resident cardiac stem cells. We will assess the arrhythmic potential of each of these types of stem cells under normoxic and hypoxic conditions, with/without application of oxidative stress or catecholamines. We hypothesize that these methods will prove to be an effective way to screen for arrhythmic risk of cardiac stem cell therapy. Ultimately, our approach can potentially be personalized to develop a robust screening protocol in order to identify which stem cell type carries the least amount of risk for arrhythmia. This system will have great clinical benefit to improve the risk/benefit ratio of human stem cell therapy for heart disease.

Keywords

Multielectrode array; stem cell; arrhythmia

Introduction

Cardiovascular Disease is the leading cause of death worldwide and will likely account for 30 percent of all deaths by 2015 (1). In the United States, approximately one American dies from cardiovascular disease every forty seconds. Heart failure accounts for more than one million hospitalizations each year in the United States and results in a \$35 billion cost to

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health care. Although there have been recent advances in the treatment of heart failure, the long-term mortality rate still remains high, at about 50% (1).

A major cause of heart failure is ischemic disease, precipitated by acute myocardial infarction (MI) leading to cardiomyocyte death. The heart has limited capacity to regenerate itself, so cardiomyocyte death typically leads to fibrosis and progressive ventricular remodeling. Stem cell therapy has emerged as a promising approach to repair the injured myocardium, and the field of cardiac regenerative therapy is rapidly advancing. Studies performed to date have shown that stem cells have the ability to partially restore contractile function and promote revascularization of ischemic regions (2). Bone marrow-derived mesenchymal stem cells (MSCs), skeletal myoblasts, and resident cardiac stem cells have all been successfully employed in clinical trials of stem cell transplantation in heart failure patients (3).

Although stem cells have great therapeutic potential, they also pose risks for adverse events. Numerous studies have shown that stem cell therapy can increase the frequency and/or severity of cardiac arrhythmias (i.e., proarrhythmia) (4-7). Ventricular arrhythmias are the most common cause of sudden cardiac death, and ventricular tachycardia in particular has been associated with cardiac stem cell therapy (8)(9). Thus far, simple and reliable tests to gauge the proarrhythmic risk of cardiac stem cell therapy have not been developed. Here, we propose to use a novel high throughput method to screen skeletal myoblast stem cells, MSCs and cardiac stem cells to assess their potential to cause arrhythmic events after stem cell transplantation.

Skeletal myoblasts have been an attractive option for stem cell therapy because they are easy to obtain directly from the patient and resistant to ischemia (2). However, after transplantation, myoblasts begin to lose the ability to express connexin and gap junction proteins (4). Myoblast stem cells have been demonstrated to cause arrhythmic events when transplanted into human subjects. In the “Myoblast Autologous Grafting in Ischemic Cardiomyopathy” (MAGIC) clinical trial, a higher number of ventricular arrhythmic events were observed early in the post-implantation period in patients who were treated with myoblast stem cells (10). The patients who developed ventricular arrhythmias were implanted with an implantable cardioverterdefibrillator (ICD). During follow-up visits, a significant number of these patients continued to experience ventricular arrhythmias despite treatment with anti-arrhythmic drugs (10). It has been hypothesized this increase in proarrhythmia manifesting as ventricular tachyarrhythmias developed due to the newly differentiated myotubes’ failure to express gap junction proteins. This impaired electrical coupling results in abnormally slow conduction velocities thereby promoting impulse reentry leading to an increased risk for arrhythmic events (11).

From a therapeutic standpoint, bone marrow-derived MSCs are advantageous due to their ability to home in on sites of injury and their lack of cell surface markers used by immune system to recognize foreign antigens (12). As such, these MSCs can be obtained from unrelated donors and used as an “off-the-shelf” form of cardiac stem cell therapy. Numerous in vitro experimental and clinical trials have been performed using MSCs with promising results. Chen et al reported that MSC transplantation resulted in significant improvements in

ejection fraction when compared to control, and these results were replicated by other studies (13). However, an increasing number of studies suggest that like skeletal myoblasts, MSCs are proarrhythmic. In vitro studies demonstrated that when mesenchymal stromal stem cells were co-cultured with neonatal rat ventricular myocytes, conduction velocity slowed and there was an increased probability of sustained re-entrant arrhythmias (5). Studies have shown that cardiomyocytes treated with mesenchymal stem cells had prolonged action potential duration (14). Similar to the in vitro data, in vivo studies have also suggested that transplanted bone marrow derived stem cells may also cause delays in electrical propagation, which may result in arrhythmia (10).

Finally, there has been an increasing interest in studying the potential of resident cardiac stem cells (CSCs) as therapeutic agents. Recent studies have demonstrated that endogenous CSCs are present in the adult heart and retain their capacity to differentiate (15). These resident CSCs offer the advantage of being optimally programmed to differentiate into cardiovascular cells and survive in myocardial tissue and thus should be prime candidates for cardiac stem cell therapy. Animal models have been used to study the impact of transplanted CSCs in ischemic heart disease, and the studies thus far have shown significant improvement in heart function over control groups (16). Very few clinical trials to date have studied the role of CSCs in cardiac regenerative therapy. One notable clinical trial is the CADUCEUS Trial, which was the first clinical trial to demonstrate recovery of heart damage following treatment with CSCs. Patients with recently reperfused MI and moderate left ventricle dysfunction received autologous cardiosphere-derived stem cells. After one year, patients who received the CSCs had decreased cardiac scar size, while the scar size in the control group remained the same. However, during the one year follow-up, 47.1% of subjects who were treated with the CSCs had at least one episode of non-sustained ventricular tachycardia, compared to only 25% of the subjects in the control group. Despite this increase in ventricular ectopy in the CSC-treated group, there were no reports of sustained ventricular tachycardia or ventricular fibrillation (17). Even if future trials demonstrate a low incidence and severity of arrhythmias related to cardiac stem cell therapy, its widespread application would likely be limited due to the risk and expense of obtaining these cells from humans for autologous transplantation.

In conclusion, evidence is mounting to suggest that stem cell transplantation has a favorable impact on heart function but may increase the risk of arrhythmias. Therefore, a critical step to improve safety of stem cell therapy is to develop a robust cell screen to identify the stem cell type(s) and/or lines that may pose a lower or absent risk of arrhythmia. We propose a novel method using a multielectrode array to identify the safest stem cell type for transplantation that could be adapted to for use in individual patients.

Hypothesis

We hypothesize that in vitro co-culture system and a multielectrode array, in combination with stress conditions that mimic disease states, such as hypoxia, oxidative stress, and catecholamines, can be a novel and effective approach to predict arrhythmic activity of transplanted stem cells in the heart in order to identify the safest type of stem cells to employ clinically.

Discussion

Many different models have been devised to study the properties of stem cells in vitro. To investigate the arrhythmic potential of stem cell transplantation, we will isolate adult cardiomyocytes and co-culture them with mesenchymal stem cells, skeletal myoblast stem cells, and cardiac stem cells. Chang et al. successfully employed a co-culture model to determine whether mesenchymal stem cells caused proarrhythmic effects when co-cultured with rat ventricular myocytes (5). Major advantages of this model include ease of manipulation of the location and quantity of stem cells and associated conditions (i.e. hypoxia, oxidative stress, and catecholamines), ability to directly compare different stem cell types under identical conditions, and the detailed analysis of electrophysiologic properties. These advantages may be partially offset by the simplistic nature of the in vitro model which excludes proarrhythmic influences related to medications, systemic electrolyte disturbances and inflammatory factors that may be present in the heart failure milieu(18).

Currently, several methodologies have been employed to measure and record electrophysiological activity in cardiac cells. Optical mapping and patch clamp recording are two such methods which are highly effective but technically demanding and low-throughput. Additionally, contractions of the cardiomyocytes in culture induce numerous artifacts in the signal that negatively impact data quality (19). While the patch clamp method is an invaluable tool to measure action potentials and the function of specific ion channels, the methodology is far removed from the in vivo state and cannot be employed for long-term measurements (20).

In our proposed study, the MED64 multielectrode array system will be used to acquire electrophysiological data. This instrument has a low-impedance planar multielectrode, which results in low-noise recordings and thus more accurate data acquisition. In contrast to the patch clamp and optical mapping methods, multielectrode arrays (MEA) are high-throughput and conducive to long-term observations of system level extracellular currents as well as cell-to-cell interactions (20). MEAs can simultaneously record electrophysiological information at a high signal-to-noise ratio and provide high-frequency electrical stimulation to a large amount of cells, without damaging the plasma membranes (21). MEAs can also examine network level activity, which includes conduction patterns and velocities (22). Since MEAs have only recently become commercially accessible, very few studies have used this method to evaluate the arrhythmogenic effects of stem cell transplantation.

As mentioned earlier, arrhythmias are a significant concern with stem cell therapy. The arrhythmic risk may be modulated by factors such as hypoxia, catecholamines, and oxidative stress present in the ischemic heart milieu (23-25). Exposing the cells to these factors in vitro while recording their electrophysiological responses may simulate the in vivo environment. As such, researchers may gain valuable insight regarding which stem cell type has the lowest probability of causing arrhythmias under certain conditions.

Hypoxic conditions must be taken into account when transplanting stem cells in subjects with severe ischemic heart disease. When considering hypoxia-induced arrhythmias it is essential to first understand the importance of the role of cell-to-cell electrical coupling.

Cell-to-cell coupling in the heart is modulated by gap junctions, which are composed of connexin proteins. The expression and degradation of connexins thus modulates cell-to-cell coupling of the heart. In heart disease, the organization of gap junctions and connexins is altered and contributes to the susceptibility of the cardiac tissue to arrhythmia (26). This leads to cell-to-cell uncoupling, and as more cells become electrically uncoupled, impulse propagation becomes slower and increasingly anisotropic thereby predisposing to reentry. In ischemia, conduction is slowed due to several factors, including changes in the excitability of cells and the magnitude of the excitatory inward currents (24). Implantation of skeletal myoblast stem cells or mesenchymal stem cells may further slow the conduction velocity in the heart to exacerbate arrhythmias. By employing hypoxic conditions in vitro, our co-culture model may provide valuable insight into the potential risks of transplanting stem cells into patients with ischemic cardiomyopathy.

Another factor to consider is catecholaminergic stimulation caused by epinephrine. Epinephrine is released from both the adrenal medulla and myocardium after a myocardial infarction, leading to significant increases in plasma and cardiac levels (27). Epinephrine has been reported to trigger arrhythmias and to increase infarct size in humans, and levels of epinephrine in the plasma are correlated with the severity of ventricular arrhythmias (28). Increased beta-adrenergic stimulation caused by epinephrine is also arrhythmogenic in chronic heart failure, particularly in the setting of hypokalemia which may be provoked by diuretic use in these patients (29).

In addition, one must consider oxidative stress and its ability to aggravate arrhythmias. One important scenario that can arise after treatment of myocardial infarction is reperfusion injury, in which cardiomyocytes that are re-oxygenated are injured by an abrupt burst of reactive oxygen species (ROS) (31). In cardiac tissue, low levels of ROS play an important homeostatic role in regulating ion channels and cellular metabolism (25). In reperfusion injury, however, high levels of ROS damage the myocardium by directly injuring the cell membrane and causing cell death. Specifically, these ROS interact with poly-unsaturated fatty acids and form hydroperoxides and lipid peroxides which can damage the sarcolemma. ROS also stimulate the recruitment of neutrophils, which act to further amplify ROS production and promote inflammation. Moreover, the increase in ROS reduces bioavailability of nitric oxide, leading to microvascular dysfunction and impaired blood flow (31).

Finally, inflammatory cytokines are produced in the ischemic tissue, which regulate cell survivability. While ROS trigger the formation of inflammatory cytokines, inflammatory cytokines can also stimulate the production of more ROS. Both ROS and inflammatory cytokines cause dysregulation of intracellular Ca^{2+} (32). Elevated ROS can also perturb sodium channels on cardiomyocytes, remodel gap junctions, and affect mitochondria function. Each of these actions can alter electrophysiological properties (25).

To date, no studies have employed a multielectrode array to screen for arrhythmias in stem-cell treated cardiomyocytes in vitro. This novel approach will expose the cell model to different experimental conditions that mimic various disease states: hypoxic conditions, treatment with epinephrine, and treatment with reactive oxygen species. Using this method,

we expect to be able to determine which stem cell type/line may be least susceptible to creating a proarrhythmic milieu following transplantation in vivo. The data gathered will increase our understanding of the risks of stem cell transplantation and ultimately lead to more favorable clinical outcomes in patients receiving stem cell therapy for heart disease.

Implications of Hypothesis

This novel screening method will help to predict which stem cell type carries the least amount of risk for arrhythmia when used to treat heart disease. Producers of bone marrow-derived MSCs for cardiac applications could employ this approach to test individual lines of cells in order to select those which have the highest safety margin for clinical use. In the future, our approach can be used to develop an efficient, personalized approach to stem cell therapy. In the clinical setting, this is expected to increase the safety of stem cell transplantation in humans with heart disease.

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We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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