Cardiac stem cell based therapy is a promising therapy for patients with severe heart failure. Many types of stem cells, such as embryonic stem cells, myoblasts, marrow-derived mesenchymal stem cells, circulating endothelial progenitor cells, and cardiac precursor cells etc, are known as cellular sources for cardiac stem cell therapy. Both in the clinical and experimental setting, stem cells are reported, and supposed, to cause some arrhythmogenic adverse effects. In order to overcome these serious adverse effects, it is necessary to know the electrophysiological properties of stem cell-derived cardiomyocytes, and have a profound insight into the mechanisms of arrhythmia to know whether such arrhythmogenic properties of the cells can cause serious arrhythmia in situ. In the present study, recent publications that focus on the electrophysiological aspect of stem cell based therapy are reviewed and, furthermore, a new perspective on cardiac stem cell therapy of arrhythmias is given. (Circ J 2007; Suppl A: A-45–A-49)

Key Words: Heart; Proarrhythmia; Stem cell; Sudden death

Skeletal Myoblast-Related Arrhythmias

Skeletal myoblasts are a major cell source for cardiac stem cell therapy in patients with severe heart failure. Autologous Skeletal myoblasts can be obtained from the patient and easily multiplied in vitro. Autologous stem cell transplantation is favorable because it does not require immunosuppressive agents to prevent rejection of engrafted stem cells. Furthermore, skeletal myoblasts are known to be resistant to hypoxia, and can be differentiated into cardiomyocyte in vitro by means of cell fusion; however, the frequency of fusion is extremely low. Although a clinical trial of autologous skeletal myoblast transplantation therapy in patients with ischemic cardiomyopathy showed a modest, but significant, recovery of cardiac function, the results were a warning of the possibility of a proarrhythmic aspect of stem cell therapy. In fact, skeletal myoblast transplantation caused significant ventricular tachyarrhythmias that required implantation of cardiac defibrillator.

Both the action potential (AP) duration and refractory period of skeletal myoblasts are extremely short compared with cardiomyocytes. Therefore, skeletal myoblasts might lead to functional block and subsequent reentrant arrhythmias when they are injected into the myocardium. However, the proarrhythmia of myoblast transplantation cannot be ascribed solely to such a mechanism, because electrical coupling between skeletal myoblasts and cardiomyocytes is rare both in vivo and in vitro. Furthermore, these cells are anatomically isolated from the host heart in situ because the engrafted skeletal myoblasts are usually encapsulated by connective tissue. Those findings suggest that the beneficial effect of skeletal myoblast implantation is not from contraction of the skeletal myoblasts but from neovascularization, paracrine or passive compression of the host myocardium. The electrical instability observed in the clinical trial may have been caused by a reentry circuit forming around the anatomical obstacle created by the engrafted myoblasts. Decreases in conduction velocity and the occurrence of spiral wave reentry have been observed in cardiomyocytes co-cultured with a monolayer of skeletal myoblasts in vitro. In that experiment, skeletal myoblasts might have acted as an anatomical obstacle for the reentrant arrhythmia.

We previously observed interesting rapid and spontaneous beating of matured skeletal myocytes in vitro. The rhythm of that spontaneous activity was basically dissociated from that of the co-cultured cardiomyocytes, suggesting no electrical communication between the cardiomyocytes and skeletal myoblasts, even though there was no fibrotic encapsulation. Because the rhythm of each of the 2 types of cells was dissociated under co-culture conditions, myoblasts might contract incidentally at the vulnerable phase of the AP of cardiomyocytes. Subsequently, we observed fibrillatory contraction of co-cultured cardiomyocytes in vitro. Such arrhythmia was completely blocked by the stretch-activated cation channel blocker. Moreover, the cardiomyocytes and skeletal myoblasts showed an entirely different
response to acetylcholine secreted from the parasympathetic nerve terminal in the heart. Cardiomyocytes have muscarinic receptors that hyperpolarize the membrane potential and stabilize automaticity, whereas skeletal myocytes have nicotinic receptors, which activate the agonist-gated Na channels and elicit APs. Therefore, parasympathetic nerve activation in transplant recipients can lead to tetanic activation of the AP of the engrafted skeletal myoblasts, which may cause ventricular arrhythmia via stretch-activated channels of the host cardiomyocytes. Thus, blockade of stretch-activated channels or autonomic blockade (not only sympathetic but also parasympathetic nerves) may help suppress the ventricular arrhythmias related to skeletal myoblasts transplantation.

MSCs and Arrhythmias

Cardiomyocytes can be generated from marrow-derived MSCs. During the maturation in vitro of murine regenerated cardiomyocytes in our previous experiment, we frequently observed an unstable (so-called “immature”) shape of the APs (Fig 1), which disappeared as the cells matured. In human MSCs, unstable APs were also observed in the early stage of maturation and they gradually became stable with time after cardiomyogenic induction. These findings suggest that immature regenerated cardiomyocytes forming immediately after transplantation of MSCs may be proarrhythmic by the mechanism of triggered activity, but that such unstable proarrhythmic action can be stabilized with maturation of the MSC-derived cardiomyocytes. Cardiomyocytes generated from engrafted MSCs in vivo were sparse and rod-shaped within the host heart and seemed to connect with the adjacent host heart muscle by intercalated disks, suggesting a tight electrical connection between the host and graft. Such a connection would stabilize the immature repolarization of MSC-derived cardiomyocytes by the electrotonic effect of the surrounding host cardiomyocytes.

Chang et al elegantly showed that co-cultivation of MSCs led to a spiral-type reentrant arrhythmia in the cultured cardiomyocytes in vitro under the conditions of a 2-dimensional (D) culture model. Accordingly, their conclusion cannot be extrapolated to arrhythmias in situ in the 3-D heart tissue.

Published reports of clinical trials of transplantation of MSCs to patients with severe heart failure are still limited and have not yet shown any severe proarrhythmic adverse effects. The discrepancy between the basic study and the clinical outcome may be based on some favorable effect of stem cell therapy on the host cardiomyocytes. Transplantation of MSCs was reported to cause neovascularization and secretion of anti-apoptotic hormonal factor to the host cardiomyocytes. Such direct effects on damaged host cardiomyocytes might suppress the development of a substrate for arrhythmia in the host heart muscle and consequently the possible proarrhythmic action of the engrafted stem cells could be masked. Because MSCs were larger than capillaries, intracoronary injection would cause multiple coronary micro-embolization and patchy necrosis of the host myocardium, which could provide a substrate for cardiac arrhythmias.

Marrow-Derived Haematopoietic Stem Cells

The definition of marrow-derived hematopoietic stem cells is unclear. In earlier papers, CD34, CD133, or c-kit (CD117) positive cells from marrow-derived cells were selected and used for the experiments. These cells have been recently defined as hematopoietic stem cells or marrow-derived mononuclear cells. There was some confusion between hematopoietic stem cells and MSCs in the early work in this field, but hematopoietic stem cells are positive for CD34 or CD45, whereas MSCs are negative for CD34 or CD45. There was no evidence of cardiomyogenesis from hematopoietic stem cells in vitro. Orlic et al reported that hematopoietic stem cells transdifferentiated into cardiomyocytes in vivo, but recent studies concluded that hematopoietic stem cells did not transdifferentiate into cardiomyocytes in vivo. Based on those findings, hematopoietic stem cells are currently believed to restore impaired cardiac function by means of angiogenesis or a paracrine effect thus the clinical trial may not show any increase in the proarrhythmic risk with hematopoietic stem cell transplantation.
Asahara et al defined EPCs as CD34-, Flk-1- and Tie-2-positive cells in the peripheral blood. These cells are similar to marrow-derived hematopoietic stem cells in relation to cell surface markers, so it is believed that they have the potential to transdifferentiate into multiple organs. Koyanagi et al reported that EPCs had the potential to transdifferentiate into cardiomyocytes, however, there are few electrophysiological data of cardiomyocytes generated in vitro.

ES Cells

ES cells are multipotential stem cells, and in vitro have the potential to become cardiac muscle. Over the period of ES cell differentiation, the cells start to beat spontaneously and show various shapes of cardiac APs corresponding to ES cell differentiation, the cells start to beat spontaneously and show various shapes of cardiac APs. This low efficiency of cardiomyogenic differentiation of ES cells is safety. Abnormalities in genes are frequently observed in developing cells in vitro might not represent the electrophysiological properties in situ, as described later. There are also certain issues to be solved regarding clinical application. One of the major concerns is ethical and another is safety. Abnormalities in genes are frequently observed in cultured ES cells and carcinoma cell-derived cardiomyocytes showed abnormalities of repolarization; however, such an experiment for developing cells in vitro might not represent the electrophysiological properties in situ, as described later.

Resident Myocardial Progenitors

Recently, several groups have reported the isolation of cardiac stem cells, so-called “cardiac progenitor cells” (CPCs). In vitro these cells are defined by the surface markers c-kit, Sca-1, an expressed the transport protein Abcg2 (so-called “side population”), or self-adherent clusters termed “cardiosphere”. Although CPCs have a high efficiency of cardiomyogenesis, their precise profile is still unclear. Also, it is not certain yet whether we can obtain sufficient cells from adult humans to repair their own damaged hearts, nor are the electrophysiological properties of cardiomyocytes generated from CPCs clearly understood.

Consideration of Models of Arrhythmia

In vitro electrophysiological experiments with heart cells or tissue immediately after isolation are popular, and are established as a method of investigating the electrophysiological properties of heart muscle in situ. However, once the heart cells are isolated, the environmental conditions are not physiological (ie, there is no blood flow or passive stretch). In our observations, culture conditions are not suitable for cardiac myocytes to maintain their normal phenotype compared with the engrafted condition. The culture medium that is commonly used might be unsuitable for stem cell-derived cardiomyocytes to maintain their electrophysiological properties. Furthermore, repolarization of the AP is very sensitive to adjacent host cardiomyocytes. Even if the repolarization of regenerated cardiomyocytes is unstable, and they show triggered activity in vitro, such arrhythmogenic properties may be prevented by the electrotonic influence of adjacent host cardiomyocytes via gap junctions.

A clinical trial revealed a proarrhythmic risk with skeletal myoblast implantation, but there are no reports of an obvious increase in cell therapy-related arrhythmic risk with other cell types. This low level of risk might be related to the low efficiency of cardiomyogenic differentiation of these cells, especially for stem cells obtained from humans. Therefore, if we were able to use stem cells with a higher potential to become a cardiomyocyte in vivo, cell therapy-related arrhythmia may become a more serious issue.

If stem cell-derived cardiomyocytes became mature in situ, the electrophysiological properties of the newly formed cardiomyocytes could be stabilized after a certain post-transplantation period. From the clinical point of view, we can monitor the occurrence of serious arrhythmia carefully in the intensive care unit during the patient’s perioperative period, and we may be able to prevent sudden cardiac death efficiently. Therefore, it is practically more important to suppress sudden arrhythmic death of patients during the remote phase after cell transplantation therapy.

Possible Application of Stem Cells for Antiarrhythmic Therapy

Biological Pacemaker Therapy

Stem cells can be used for treatment bradyarrhythmias. Make et al showed that overexpression of a dominant negative mutation in a channel gene, Ik1, generated a pacemaker-like slow depolarization potential in ventricular myocytes, and also showed acceleration of ventricular ectopy in vivo. Although they clearly showed a novel strategy for the treatment of bradyarrhythmias, the issues of durability and control of transfection of gene expression are still unsolved.

On the other hand, stem cell-derived cardiomyocytes usually show pacemaker potential and spontaneous beating activity. Thus, it is possible to transplant stem cells into the heart to treat bradyarrhythmias. From the clinical point of view, it is important to obtain regenerated cardiomyocytes with a stable pacemaker potential; however, it is speculated that the AP of generated cardiomyocytes will change with cardiomyogenic induction in vitro. Therefore, cardiomyocytes with a sinus node-like AP in the early phase of cardiomyogenic induction might change to that of a working cardiomyocyte-like AP (without pacemaker potential) over time. However, we hope that there may be a certain population of stem cell-derived cardiomyocytes with stable pacemaker activity, because physiological sinus node cells maintain stable pacemaker activity in situ. Therefore, if in the future we could obtain regenerated cardiomyocytes with stable pacemaker activity, we would be able to create a biological pacemaker.

Atrioventricular (AV) Block

Beeres et al reported that in a cultured cardiomyocytes experiment 2 separate groups of cultured cells were electrically dissociated, but connected by transplantation of marrow-derived MSC in vitro. This experiment suggested a possible strategy for treating patients with AV block. Marrow-derived MSC usually express connexin 43, so these 2 groups of cardiomyocytes could be passively connected because the cardiomyogenic differentiation ability of MSCs is extremely low. Choi et al reported that transplantation of a scaffold-based tissue of skeletal myoblasts between the AV groove restored the AV connection in an animal model of
AV block. If we had an appropriate cell source with high cardiomyogenic potential for obtaining enough regenerative cardiomyocytes and a cell sheet of regenerative cardiomyocytes, the patient with AV block might be able to be treated by this type of stem cell therapy guided by the tissue engineering in the future.

References


