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EDITORIAL

Stem Cell Therapies for Cardiovascular Diseases: What Does the Future Hold?

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Cardiovascular disease (CVD) is a leading cause of morbidity and mortality around the globe. In Australia, one in six people are affected by CVD and a total of 4.2 million people are suffering from heart disease, stroke or vascular disease. Coronary artery disease affects around 1.2 million Australians, many of whom develop chronic heart failure as a result of ischaemic cardiomyopathy. The management of CVD has evolved tremendously in the past three decades, but the majority of treatments are not curative. Pharmacotherapy, percutaneous coronary intervention and coronary bypass grafting are important therapeutic measures, but they are unable to repair the damaged myocytes or vascular structures. Stem-cell based therapies are designed to regenerate myocardium, and attenuate or reverse the remodelling of vascular structures; therefore they may fundamentally address the structural damage or cellular degeneration in CVD. For these reasons, there have been a significant number of preclinical and clinical studies conducted in this area in recent years.

In a recent article published in Heart, Lung and Circulation, we reported that when bone marrow derived mesenchymal stem cells (BMSCs) were modified by transduction with lentiviral vectors expressing eNOS or a mutant caveolin-1 protein, they produced higher levels of nitric oxide in the systemic circulation of a rat model with pulmonary hypertension (PAH) [1]. The genetically modified BMSCs also inhibited proliferation of vascular smooth muscle cells, ameliorated ultrastructural abnormalities in lung tissues, and reduced pulmonary pressure by an average of 17 mm Hg. More importantly, treatment with BMSCs producing nitric oxide for a period of 35 days was associated with a significantly higher survival rate than the animals treated with placebo [1]. These results represent a major advance in the field of stem cell therapy for CVD. However, many questions remain. What is the most effective cell type for treatment? What are the most efficient modes of delivery for stem cells? Can genetically modified stem cells be safely used in human subjects?

To date, many studies describing stem cell therapies in CVD have focussed on heart failure secondary to coronary artery disease or ischaemia. Various types of stem cells have been investigated in animal models and in human subjects, in order to suppress left ventricular remodelling and to improve cardiac function. Embryonic stem cells, induced pluripotent stem cells (iPS), skeletal myoblasts, BMSCs, and cardiac stem cells have all been found to be effective in treating heart failure in a number of experimental studies [2–10]. The most studied cell types are the BMSC, which, when directly injected into the epicardium or infarct scar tissue in the left ventricle, allogeneic BMSCs have been shown to differentiate into smooth muscle cells and endothelial cells, resulting in a reduction in infarct size, an increase in vascularity in the myocardium and an improvement in cardiac function [4–6]. Among the reported types of stem cells, the cardiac stem cells appear to be the most promising. Once delivered to the myocardium through intracoronary or intramuscular approaches, these cardiac stem cells are able to differentiate into cardiomyocytes [9]. In animal models with myocardial infarction, cardiac stem cell based therapies have attenuated ventricular remodelling and improved cardiac function [9].

The other area of active research is stem cell therapy for PAH. Endothelial progenitor cells and mesenchymal stem cells have been used to treat monocrotaline-induced PAH in animal models with various success [1,2]. The reported benefits of stem cell therapy in the PAH animal models have been inhibition of pulmonary vascular smooth muscle cell

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proliferation, reduced intima-media thickness in the pulmonary arterial walls, less collagen fibres in the lung tissues, and improved pulmonary haemodynamics in both short- and medium-term (e.g. six months) [2]. Nitric oxide synthase-transfected endothelial progenitor cells have also been trialled in a small number of patients with PAH [10]. It did not lead to sustained haemodynamic improvements at three months, but interestingly, the six-minute walk distance was increased within the first six months of the treatment [10].

Genetic modification of stem cells may improve stem cell function and survival post transplantation and a number of gene therapeutic approaches have been used in animal models of CVD such as naked plasmid DNA, adenovirus and adeno associated viral vectors (AAV) and also retroviral/lentiviral vectors. Each system has inherent limitations and safety concerns such as inflammatory response (adenoviral), restricted transgene packaging size (AAV) and random chromosomal integration (retroviral/lentiviral). A promising therapeutic approach for CVD is direct reprograming of cardiac fibroblasts to cardiomyocytes, an elegant study by Ieda et al. [11] demonstrated *in vitro* conversion of fibroblasts to functional induced cardiomyocytes (iCMs) through retroviral/lentiviral expression of three developmental transcription factors, Gata4, Mef2C, and Tbx5 (GMT). A further study demonstrated that direct viral vector mediated delivery of GMT to infarcted mouse myocardium resulted in reprogramming of target cells to cardiomyocytes, and improved cardiac ejection fraction and decreased scar size [12]. The safety profile of lentiviral vectors has recently been demonstrated in recent clinical trials [13]

The number of clinical trials using stem cell therapies to treat CVD has gathered pace over the past five years, and a large number of future trials are in the pipe line. Similar to previous experimental studies, most of the recent clinical studies on stem cell therapies for CVD have been centred on cardiac regeneration in heart failure populations. The trial results on ischaemic cardiomyopathy in humans have been inconsistent. Intracoronary infusion of cardiac stem cells that were harvested from the endocardium was found to increase left ventricular ejection fraction 4 and 12 months after the treatment [14]. Cardiosphere-derived stem cell therapies in patients with myocardial infarction were associated with a reduction in the size of infarct 12 months after the treatment, but it did not improve the cardiac function or heart failure symptoms [15]. Earlier studies on bone marrow mononuclear cells delivered through myocardial or intracoronary approach showed an improvement in left ventricular function within the first 12 months of treatment [16]. However, the promising results from the earlier studies were not reproduced by other investigators [17]. Bone marrow mesenchymal cells, when injected directly into the endocardium, were associated with a better six-minute walk test, but did not improve left ventricular ejection fraction in patients with ischaemic cardiomyopathy [18].

The mixed results from the previous clinical trials are largely due to the diversity in the type of stem cells used, mode of delivery, small trial sizes and the selection of patients. Recently, Patel and colleagues [19] reported the results of a double blind and randomised trial on 109 patients with class III or IV heart failure secondary to ischaemic cardiomyopathy. Stem cells (mostly mononuclear cells) were extracted from patient's own bone marrow, and were delivered to the myocardium through percutaneous catheterisation. After 12 months follow-up, the composite primary end point, such as death or hospitalisation for cardiovascular problems, was 38% in patients treated with stem cells, and 49% in those treated with placebo. These results are promising as this is the first piece of evidence that stem cell therapies offer some survival benefits in patients with ischaemic cardiomyopathy.

There are a number of unresolved issues involving the use of stem cell based therapies for CVD that require further investigations. Firstly, what is the ideal cell type for the treatment of CVD, in particular, ischaemic cardiomyopathy. Several ongoing trials are investigating feasibility, safety and clinical efficacy of other types of stem cells, such as mesenchymal precursor cells [20], induced pluripotent stem cells [21,22,23] or cardiopoietic stem cells [21,22,23]. However there have been no studies to compare the efficacy of different cell types in the treatment of CVD. Genetically modified bone marrow mesenchymal cells that express nitric oxide synthase have been recently reported [1,24,25]. These stem cells were able to reduce pulmonary pressure and improve 35-day survival in rats with drug-induced pulmonary hypertension. However, the efficacy of these novel nitric oxide producing cells in treating ischaemic cardiomyopathy is yet to be studied, and their safety in human subjects is unknown.

The second issue is the dosing of stem cells and their frequency of administration in various types of CVD. Almost all clinical studies today have utilised only one-off treatment protocols with stem cells and the dose of administered cells varies tremendously between trials. One study found that endocardial delivery of 20 million, 100 million, or 200 million bone marrow mesenchymal stem cells improved patient's exercise tolerance, quality of life and ventricular remodelling [18], but the dose-response relationship in this study was unclear. Third, the optimal mode of stem cell delivery is yet to be determined. Transendocardial and intracoronary deliveries have been used in most of the clinical trials, but it is unknown which delivery system is more superior. Intracoronary infusion of stem cells is relatively easy to perform. In a preclinical study, continuous and multi-coronary delivery of allogeneic cardiosphere-derived cells offered a better histological and functional outcome than a single intracoronary delivery [26]. However, the feasibility and safety of this new mode of intracoronary delivery are yet to be investigated in humans. The drawback of intracoronary cell infusion is that it is not possible to deliver the cells to the areas where a coronary artery is completely occluded. Furthermore, there is a rapid cell loss after intracoronary infusion which may have a negative impact on the efficacy of the stem cell treatment [27]. Transendocardial injection can directly deliver stem cells to the ventricular walls, in particular the areas