

The Membrane particles derived from Mesenchymal Stem Cells by Endothelial Regeneration

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Mesenchymal stromal cells (MSC) are studied as an immunomodulatory and regenerative therapy in organ repair. Recent work demonstrated that dead MSC which are unable to secrete factors, are effective in a sepsis model, suggesting that MSC activity is dependent on the cell membrane interactions with immune cells. We propose a new therapy based on the generation of plasma Membrane Particles (MP) from MSC. We previously showed that MP were effective in reducing the inflammatory phenotype of monocytes. In this study we investigated the therapeutic potential of MP as regenerative treatment for EC in a TNF- α inflammatory condition. MP were generated from MSC by hypotonic shock and extrusion. MP showed vesicle shape (cryoelectron-microscopy) and a size below 200 nm. Uptake of MP by EC was analyzed by confocal microscopy, and within 24 hours >90% of EC have taken up MP. Three different concentrations of MP were tested on EC. None of the MP concentrations induced apoptosis or activation of EC measured by the expression of the adhesion markers such as ICAM-1, and VCAM. With respect to the regenerative capacity of EC after MP treatment, we have observed an enhancement of angiogenesis by increasing the number of tubes, and branches formation *in vitro* compared to the negative control (non-treated EC). In the scratch wound healing assays, MP had a stimulating effect on EC to fill the scratch in a dose-dependent manner. In conclusion, MP potentially serve as a novel cell derived therapy that restores vascular integrity and induces endothelial regeneration. The prevalence of ischemic heart failure remains markedly high despite several recent therapeutic advances in the treatment of acute myocardial infarction. Alternative pharmacological treatments are being developed for the failing heart, but the mortality and quality of life in advanced stages of congestive heart failure are still significant issues. Recently, stem cell-based therapy has emerged as a promising treatment of severe postinfarction systolic left ventricular dysfunction, because stem cells are capable of differentiating into cardiomyocytes, cell grafting within the damaged myocardium may theoretically limit the consequences of the loss of contractile function. Another proposed effect of transplanting stem cells involves augmentation of angiogenesis and consequent improvement of myocardial ischemia. Preclinical studies investigating bone marrow-derived cells as treatment for ischemic myocardium have been performed preferentially in the acute ischemia model; data in the chronic ischemia model are lacking. Moreover, chronic myocardial ischemia leading to heart failure is a leading cause of morbidity and mortality in the United States. Accordingly, the aim of the present study was to determine whether bone marrow-derived MSC transplantation

would improve the morphology and function of the heart in a chronic canine model of myocardial ischemia.

This study was reviewed and approved by the University of Texas Health Science Center Houston (UTHSCH) Animal Welfare Committee and conducted at the UTHSCH Center for Laboratory Animal Medicine and Care, located at the Department of Veterinary Medicine and Surgery of the University of Texas M.D. Anderson Cancer Center. For this study, 12 healthy adult mongrel dogs of either sex, weighing between 25 and 35 kg each, were subjected to open-chest surgery. The present study describes MSC transplantation in a canine model of chronic ischemia. The results suggest that injecting MSCs into ischemic myocardium results in improved myocardial function and increased vascularity. In experimental studies, bone marrow-derived cells have been shown to regenerate areas of infarcted myocardium and coronary capillaries,¹⁴ thus limiting functional impairment after myocardial infarction. Transendocardial injection of autologous bone marrow mononuclear cells has been shown to increase myocardial contractility and perfusion in swine.⁷ Various cell lineages have been used to generate evidence that bone marrow stem cells differentiate into cardiomyocytes, endothelium, and smooth muscle cells.^{15,16} However, there is much controversy regarding which stem cell subtype might be responsible for the therapeutic benefit of bone marrow mononuclear cell transplantation into ischemic myocardium.⁵ The bone marrow mononuclear cell subset, which is quite heterogeneous, is composed of MSCs, hematopoietic progenitor cells, endothelial progenitor cells, and more committed cell lineages such as natural killer lymphocytes, T lymphocytes, and B lymphocytes. In theory, the ideal cell type for cellular therapy is likely to be a less committed one that can undergo full cardiomyocyte differentiation, augment angiogenesis, and trigger vasculogenesis. In that regard, MSCs may have the necessary combination of plasticity¹⁷ and viability.

In vitro, MSCs are capable of transdifferentiating into functional cardiomyocytes under differentiation-inducing culture conditions (eg, when treated with 5-azacytidine).¹⁸ *In vivo*, it has been shown that, in the acute ischemia model or in a myocardial environment, MSCs differentiate into cardiomyocyte-like cells that express desmin, troponin T, and sarcomeric MHC and produce a concomitant functional benefit.^{10,16} However, *in vivo* transdifferentiation of hematopoietic stem cells into cardiomyocytes after cell transplantation remains an object of controversy.¹⁹ In the present study, colocalization of MSCs with cardiac muscle-specific proteins was not observed. This might be because of a

lack of specific cell signaling in the chronically ischemic myocardium. In the setting of chronic ischemia, the tissue injury cascade and compensatory response are different from that present in acute myocardial infarction. Rapid and massive activation of the inflammatory cascade, which is characteristic of acute myocardial infarction and responsible for inflammatory cell infiltration, may be required for MSC myogenesis. It is particularly intriguing, however, that quantitative morphometry revealed a trend toward reduced fibrosis in the anterolateral wall of stem cell-treated hearts compared with controls ($P=0.08$). The major limitation of the present study is the small number of animals in each group, which limits conclusions about efficacy. However, statistically significant differences between the treatment and control groups were shown in regard to echocardiographic parameters and capillary density. In addition, one limitation of our chosen model that could, in theory, limit interpretation of cardiac function data is the fact

that dogs might develop substantial collateral circulation. However, coronary angiography was performed both before and 30 days after cell and saline injection to ensure the correct placement of the ameroid constrictor and to assess for the development of new collateral vessels. No significant differences in major collateral development were seen between treated and control hearts. However, nonvisible collaterals may have been present.

In conclusion, the present study suggests that implantation of MSCs into chronically ischemic myocardium is safe and effective. MSCs differentiated into smooth muscle cells and endothelial cells, resulting in increased vascularity and improved cardiac function. Pending future studies with larger sample sizes, the present findings suggest that MSC transplantation might one day become an alternative therapy for ischemic heart failure.