Effects of Long-Term Sustained Elevation of cGMP Levels and Stem Cell Implantation on Cell Death and Fibrosis in the Heart after Acute Myocardial Infarction in the Rat

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A heart attack occurs by interruption of blood supply to the heart, usually secondary to occlusion of a coronary artery by the rupture of an atherosclerotic plaque. Lost cardiac cells (cardiomyocytes) are replaced by fibrotic scar tissue, namely collagen fibers, profibrobic factors, and myofibroblasts, the key cells in fibrosis. Diabetes mellitus is a major risk factor for coronary disease and patients suffering from diabetes demonstrate a greater prevalence of heart attacks compared to the general population.

There is considerable interest in exploring a variety of cells that can differentiate and replace heart cells lost as a result of the heart attack. Specifically, investigators wish to utilize implanted stem cells or pharmacologically stimulate dormant endogenous stem cells to restore the cardiac cells.

An animal study is ongoing to determine whether the pharmacological long term elevation of cGMP levels, through treatment with sildenafil, may stimulate the formation of cardiac cells after a heart attack, presumably by the activation of endogenous stem cells. Furthermore, muscle derived stem cells were implanted into heart tissue, to determine whether cardiac cell regeneration would ensue.

This study includes 3 groups of rats that were subject to coronary artery ligation, inducing a heart attack. Echocardiography was performed before and after arterial ligation. Groups were sacrificed at 1 and 4 weeks, and coronary tissue collected for investigation by immunohistochemistry. This report aims to provide a preliminary assessment of the degrees of cardiac scarring after a heart attack, using Picrus Sirius Red for collagen fibers, TUNEL for programmed cell death (apoptosis), and alpha smooth muscle actin for myofibroblast content.

Background

Acute myocardial infarction, commonly known as a heart attack, has been the leading cause of death in the United States for the past 80 years. Nearly one million people die each year from heart attacks or other cardiovascular diseases (eg, stroke). Of the overall population of individuals suffering from heart attacks, diabetes mellitus is a major contributor to myocardial infarction.

Studies show that 65% of those who have diabetes die from some type of cardiovascular illness. High glucose levels, or hyperglycemia, cause fatty material to accumulate inside the blood vessels. With time, as the fatty material accumulates, the material begins to harden, which could potentially lead to a blood clot or interruption in the blood flow, causing a heart attack.

However, various studies indicate that sildenafil, a type of drug used to treat erectile dysfunction, is cardioprotective. Sildenafil, commonly referred to as a PDE5 inhibitor, is beneficial in elevating the levels of cGMP, which relaxes the smooth muscle, increasing blood flow in the blood vessels. Therefore, in this study, we aimed to determine the pharmacological effects of sildenafil on the elevation of cGMP levels to stimulate *in vivo* the formation of cadiomyocytes, or heart muscle cells, by activation of endogenous stem cells after an acute myocardial infarction.

Methods and Materials

Twenty four Fischer 344 male rats were used for this study. These rats were retired breeders from a larger ongoing study. All of the rats used in this study were normal, with no illnesses or abnormalities. The rats were divided into three major groups with eight rats per group: 1) control group, no oral treatment of sildenafil and sacrificed at one week; 2) control group, no oral treatment of sildenafil and sacrificed at five weeks; and 3) treated with oral sildenafil in water to elevate cGMP levels and sacrificed at 5 weeks.

At the beginning of the experiment, echocardiographs were taken for each group of rats. After the first echocardiographs were taken, ligation of the left coronary artery was performed on the rats of each group. Immediately after surgery a second echocardiograph was taken at 4–7 days, and the rats for the first group were sacrificed at 7 days. Treatment was administered as indicated to the third group.

At the end of the 5-week period for both the second and third group, a third echocardiograph was taken and the rats for both groups were sacrificed. The left ventricle of the heart of every rat in each group was taken after the sacrifice and divided into four sections, in which paraffin embedded sections were obtained.

By examining these sections, we were able to make these histochemical determinations: 1) the acute myocardial

infarction scar (infarction size) through intense Pico Sirius red staining for collagen deposition and quantitave analysis, 2) apoptotic index through TUNEL protocol and quantitative analysis to measure the programmed cell death, and 3) the use of troponin staining as a marker for cardiomyocyte integrity.

RESULTS

In the Pico Sirius red staining, there was a moderate fibrosis in the left ventricle concentrated in the infarction zone, which was not reduced by sildenafil. We also found a moderate impairment to the cardiac ejection fraction (EF) in the heart ultrasound taken at 3–7 days after surgery. The ejection fraction was maintained until 4 weeks after ligation, in which the EF impairment was not improved by sildenafil.

DISCUSSION

Long-term continuous treatment with sildenafil failed to prevent scar formation in the infarcted heart. The results are in contrast to the cardiac and penile protective effects illustrated with slightly longer and higher dose treatment in diabetic-model rats. These results are preliminary and need confirmation by other procedures on the available specimens.

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RESOURCES

Passier R, van Laake LW, Mummery CL. Stem-cell-based therapy and lessons from the heart. *Nature*. 2008;453(7193):322–329.

Schäfer R, Northoff H. Cardioprotection and cardiac regeneration by mesenchymal stem cells. *Panminerva Med*. 2008;50(1):31–39.

Roccio M, Goumans MJ, Sluijter JP, Doevendans PA. Stem cell sources for cardiac regeneration. *Panminerva Med*. 2008;50(1):19–30.

Murphy E, Steenbergen C. Cardioprotection in females: a role for nitric oxide and altered gene expression. *Heart Fail Rev*. 2007;12(3–4):293–300.

Bolli R. Preconditioning: a paradigm shift in the biology of myocardial ischemia. *Am J Physiol Heart Circ Physiol*. 2007;292(1):H19–27.

Jones SP, Bolli R. The ubiquitous role of nitric oxide in cardioprotection. *J Mol Cell Cardiol*. 2006;40(1):16–23.

Boudina S, Abel ED. Diabetic cardiomyopathy revisited. *Circulation*. 2007;115(25):3213–3223.

An D, Rodrigues B. Role of changes in cardiac metabolism in development of diabetic cardiomyopathy. *Am J Physiol Heart Circ Physiol*. 2006;291 (4):H1489–506.

Ramos GA, Hare JM. Cardiac cell-based therapy: cell types and mechanisms of actions. *Cell Transplant*. 2007;16(9):951–961.

Kovanecz I, Ferrini MG, Vernet D, et al. Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes melittus. *BJU Int*. 2006;98:116–124.

Kovanecz I, Nolazco G, Ferrini MG, et al. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus. *BJU Int*. submitted 2008.

Ferrini MG, Kovanecz I, Sanchez S. Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. *Biol Reprod*. 2007;76: 915–923.

Artaza JN, Reisz-Porszasz S, Dow JS, et al. Alterations in myostatin expression are associated with changes in cardiac left ventricular mass but not ejection fraction in the mouse. *J Endocrinol*. 2007;194(1):63–76.

Kovanecz I, Rambhatla A, Ferrini MG, et al. Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res*. 2007;100(4):867–874.

Rambhatla A, Kovanecz I, Ferrini M, et al. Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: experimental and clinical review. *Int J Impot Res*. 2008;20(1): 30–34.

Artaza JN, Singh R, Ferrini MG, et al. Myostatin promotes a fibrotic phenotypic switch in multipotent C3h 10T1/2 cells without affecting their differentiation into myofibroblasts. *J Endocrinol*. 2008;196:235–249.