Microparticle-mediated Nox2-siRNA therapy for preventing cardiac dysfunction following myocardial infarction

Inthirai Somasuntharam, Jay C Sy, Gokul Seshadri, Niren Murthy and Michael Davis
Department of Biomedical Engineering, Georgia Institute of Technology and Emory University

Statement of Purpose

Heart failure is the leading cause of death in the developed world, and myocardial infarction (MI) is the most common cause with 935,000 recurrent and new cases annually. Following MI and subsequent loss of cardiomyocytes, the myocardium undergoes structural remodeling which includes inflammatory reactions and scar formation leading to impaired ventricular function. Production of reactive oxygen species (ROS) is a key event involved in this pathogenesis [1]. Oxidative stress at high levels lead to many of the injury associated changes: proinflammatory cytokine release, cardiomyocyte apoptosis, matrix deposition, fibroblast proliferation and hypertrophy. Nicotinamide adenine denucleotide phosphate (NADPH) oxidase with Nox2 as the catalytic subunit, is a major source for cardiac ROS production [1]. After MI, NADPH oxidase expression is significantly increased in the infarcted myocardium, primarily in neutrophils, macrophages and myocytes[2]. Moreover mice lacking the Nox2 gene are protected from ischemic injury[1]. Chronic antioxidant treatment following MI in animal models improves myocyte survival, attenuates ventricular remodeling, and leads to partial preservation of left ventricular function. While chronic antioxidant therapy may be feasible, other oxidases may serve important functions in homeostatic balance. Therefore silencing the catalytic subunit of NADPH oxidase Nox2 is an exciting potential therapeutic target to improve function and prevent heart failure.

RNA-mediated silencing (siRNA) of gene expression holds great promise as therapeutics owing to high specificity and potency. However, poor serum stability of negatively charged siRNA, membrane impermeability and sequestration by lysosomes upon endocytosis have limited effective delivery. We propose to use a new class of acid-degradable polymers, polyketals[3], as delivery vehicles for Nox2-siRNA to the post-MI environment. Polyketals have controllable release kinetics and neutral degradation products. Published studies from our laboratory demonstrate that microparticles are retained in the myocardium following injection and are stable at neutral pH levels. When engaged by macrophages, present in high quantities during MI, particles are taken up and contents released within cells in active form. Polyketal PK3 was chosen for this application due to its fast hydrolysis rate

Methods: Double stranded Nox2-siRNA (mouse) (sense strand: 5'CCA UUC GGA GGU CUU ACU UUU-3') and scrambled siRNA (sense strand: 5'-GCU ACU CCG UUG CUA UUG AUU-3') were synthesized by Dharmacon. PK3 was synthesized as described in Sungmun Lee *et al[3]*. Fluorescent (FI)-Nox2-siRNA ion-paired to the cationic lipid DOTAP was encapsulated by PK3 via an oil/water single emulsion procedure. Particle size and shape were determined by scanning electron microscopy (SEM). Loading efficiency was calculated by hydrolyzing the particles and measuring the fluorescence of released FI-siRNA using a fluorescent plate reader. In vitro studies were conducted using RAW264.7 macrophage cell line.

Results: The number average molecular weight of PK3 is 2316 Daltons as determined by gel permeation chromatography using a Shimadzu system. Spherical shaped particles averaging 800nm diameter were confirmed by SEM (fig 1, left). Average loading efficiencies calculated for FI-Nox2-siRNA and FI-scrambled-siRNA were 35% and 65%, respectively. Macrophages treated at a concentration of 72nM FI-Nox2-siRNA, FI-scrambled-siRNA or empty particles containing DOTAP demonstrated a 40-50% knockdown in Nox2 gene expression in Nox2-siRNA treated samples compared to controls at days 1 and 2 analyzed by RT-PCR.

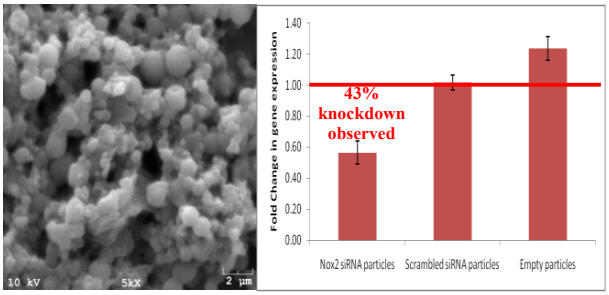

Fig1. (left) Representative 5kX SEM image of FI-Nox2-siRNA PK3 particles. (right) Fold changes in Nox2 gene expression 24 hrs post particle treatment

Preliminary studies to demonstrate functional efficacy of siRNA delivery are underway utilizing a dihydroethidium dye based fluorescent plate reader method to assay Nox2 activity in treated cells. Initial experiments demonstrate a reduction in phorbol 12-myristate 13-acetate (PMA)-stimulated Nox2 activity upon FI-Nox2-siRNA treated samples compared to controls. Results will be confirmed using quantitative HPLC.

Conclusions: Following MI, there is a robust inflammatory response involving phagocytic cells (neutrolphils, monocytes, macrophages) that are traditionally hard to deliver siRNA to. While many studies show Nox2 is a possible therapeutic target, to date no successful siRNA therapy has been reported. Our preliminary studies demonstrate that Nox2 siRNA can be encapsulated within polyketal particles, which serve as excellent delivery vehicles for macrophages. Successful completion of these studies could lead to a novel treatment for post-infarction injury and to large animal testing of polyketal particles for treating myocardial infarction.

References:

1.Zhao, W. Cardiovasc Pathol, 2009. **18**(3): p. 156-66.
2.Krijnen, P.A.J Clin Pathol, 2003. **56**(3): p. 194-9.
3.Lee, S. Nucleic Acids Res, 2009. **37**(22): p. e145.