Polyketal-mediated drug and protein delivery for cardiac regeneration

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Introduction: Cardiovascular disease is the leading cause of mortality in the United States and affects over 70 million in various age ranges. Acute myocardial infarction patients are traditionally treated at the hospital with blood thinning agents and/or angioplasty to try and clear the affected vessels. Local cell death that occurs during this acute period leads to chronic heart failure, marked by increased ventricular size and reduced contractile function. Currently, the only treatment for heart failure is transplantation surgery. It is estimated that less than 30% of transplant patients survive to receive their new heart. Thus, finding a way to limit the initial damage could potentially prevent the onset of contractile dysfunction and become the frontline treatment in patients presenting with myocardial infarction.

Myocardial dysfunction is usually progressive and successful therapy will likely require sustained delivery. Small molecule inhibitors and antioxidant proteins have great clinical potential in the treatment of cardiac disease, but their size and stability make them difficult to target to the myocardium. SB239063 has been successful in preventing cardiovascular dysfunction but treatment protocols are prolonged and may not translate to larger animals. Additionally, superoxide dismutase (SOD) may be quite beneficial to the infarcted myocardium, but cell permeability and a plasma half-life of 2 hours make treatment options untenable. Polyketal (PK) particles are a new class of biomaterials that hydrolyze slowly at physiological pH values and degrade to non-toxic compounds. Because of these positive characteristics, we hypothesized that polyketal particles represented an ideal delivery mechanism for sustained inhibition with only one injection.

Results: Here we show that in vitro, TNF-α increased p38 phosphorylation in cultured macrophages, and polyketal-encapsulated SB239063 (PKSB) time-dependently inhibited p38 phosphorylation. In addition, PKSB, and not empty polyketals (PK), inhibited TNF-α stimulated superoxide production as measured by accumulation of superoxide-specific product of dihydroethidium, 2-hydroxyethidium (con=0.14μM; TNF=0.82μM; PKSB=0.37μM; $p<0.05$). To determine efficacy in vivo, we first established by skeletal muscle injection studies that polyketal treatment did not result in inflammation in mice as measured by staining for CD45.

We then performed a randomized and double-blinded study in rats subjected to myocardial infarction. Immediately following coronary artery ligation, rats were injected with PK, PKSB, or free SB239063 intramyocardially. Three days following infarction, there was a significant reduction in p38 phosphorylation within the infarct zone of PKSB rats, with no effect of PK or the free inhibitor. In addition, only PKSB attenuated infarct-zone superoxide production (MI=27.68μM; PKSB=8.49μM; $p<0.05$) and TNF-α production (MI=157.73 pg/mg; PKSB=103.60 pg/mg; $p<0.05$). In a separate randomized and double-blinded study, we examined cardiac function by MRI and echocardiography at 7 and 21 days post treatment. While there was no difference at 7 days post-infarction, PKSB, and not PK or SB239063 significantly inhibited progression of dysfunction at 21 days. In addition, we stained tissue sections from rats at 21 days for collagen using Sirius Red staining. There was excessive scar formation in rats subjected to myocardial infarction (>50% of the left-ventricle). There was no significant affect of the empty polyketals or free inhibitor, but PKSB significantly reduced collagen staining.

In addition using a double-emulsion technique, we were able to encapsulate SOD with 25% efficiency PK microparticles (PK-SOD). To determine superoxide scavenging capability of PK-SOD, we measured superoxide production of PMA-stimulated macrophages in the presence of either empty microparticles (PK) or PK-SOD. We found a 1.6-fold increase in extracellular superoxide in PMA-stimulated macrophages. While there was no effect of PK (1.9-fold increase), PK-SOD dose-dependently scavenged extracellular superoxide production (1.1-fold increase). Additionally, PMA produced a 3.9-fold increase in intracellular superoxide that was not reduced by free SOD protein (3.4-fold increase) or PK (3.7-fold increase), but dose-dependently inhibited by PK-SOD (1.5-fold). This increase in superoxide scavenging by PK-SOD also resulted in a decrease in LPS-induced TNFα and IL-6 production.

Conclusions: In summary, our data demonstrate that SB239063 retains its function following encapsulation and is able to deliver the inhibitor to macrophages in vitro. This results in an inhibition of p38 signaling and downstream effector activation. In vivo, the PKs did not induce an inflammatory response when compared to PLGA, a commonly used substrate with acidic degradation products. Following myocardial infarction injury, PKSB performed significantly better than the free inhibitor or the control empty polymer in reducing p38 phosphorylation and superoxide production. This led to a gradual improvement in cardiac function from 7 to 21 days following injury. This improvement may be attributed to a decrease in cardiac fibrosis as measured by collagen staining.

In addition to the small molecule inhibitor studies, we also found that polyketals particles could be used to encapsulate SOD protein for both intra- and extracellular superoxide scavenging. This may play a critical therapeutic role for diseases involving increased oxidative stress. Thus, we conclude that polyketal encapsulation is a novel approach for delivering a variety of molecules for cardiac regeneration.