Protein kinase C beta II (PKC βII) peptide inhibitor exerts cardioprotective effects in myocardial ischemia/reperfusion injury

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Introduction
Coronary heart disease is the leading cause of death worldwide, and is primarily attributable to the deleterious effects of tissue injury after an ischemic insult. The most effective therapeutic interventions for reducing infarct size associated with myocardial ischemia injury is timely and effective reperfusion of blood flow back to the ischemic heart tissue. However, the reperfusion of blood itself can induce additional cardiomyocyte death that can account for up to 50% of the final infarct size. Currently, there are no effective clinical pharmacologic treatments to limit myocardial ischemia reperfusion (MI/R) injury in heart attack patients [1]. Reperfusion injury is initiated by decreased endothelial-derived nitric oxide (NO) which occurs within 5 min of reperfusion [2], and may in part be explained by PKC βII mediated activation of NADPH oxidase, which occurs upon cytokine release during MI/R [3]. PKC βII activity is increased in animal models of MI/R injury [4, 5]. PKC βII is known to increase NADPH oxidase activity in leukocytes, endothelial cells and cardiac myocytes via phox47 phosphatase and decreases eNOS activity via phosphorylation of Thr 495 [6, 7, 8]. NADPH oxidase produces superoxide (SO) and quenches endothelial derived NO in cardiac endothelial cells. Moreover, PKC βII phosphorylation of phospholipase C at Ser 36 leads to increased mitochondrial reactive oxygen species (ROS) production, opening of the mitochondrial permeability transition pore (PTP), and proapoptotic factors leading to cell death and increased infarct size [9] (fig 1). Therefore, using a pharmacologic agent that inhibits the rapid release of PKC βII mediated ROS, would attenuate endothelial dysfunction and downstream proapoptotic pathways when given during reperfusion and should be an ideal candidate to attenuate MI/R injury. In the current study, we generated MI/R injury by inducing global ischemia for 30 min. in animal models of MI/R initiated by myocardial ischemia reperfusion (MI/R) processes in MI/R injury.

Hypothesis
We hypothesize that PKC βII peptide inhibitor will improve postischemic cardiac function and reduce infarct size in isolated perfused rat hearts (ex vivo) subjected to global MI/R compared to non-drug control I/R hearts in both MI/30min & MI/30min/80min studies.

Methods
Isolated Rat Heart Preparation
Male Sprague Dawley (SD) rats (275-325g) were anesthetized i.p. (pentobarbital sodium 60 mg/kg and 1,000U of sodium heparin). Hearts were rapidly excised and perfused at a constant pressure of 80 mm Hg with a modified physiological Krebs’ buffer aerated with 95% O2 and 5% CO2, maintained at 37° C and pH 7.4 by automatic HBOS WP3000s (Harvard Apparatus, MA). A 1000U/mL of COLUNGEN (Shandon, PA) was added to the perfusate to prevent heart muscle contracture. hearts were perfused in the presence of the following drugs: 100 µM . The buffer was delivered at 10 mL/min to the hearts using a peristaltic pump (Model 2200,Harvard Apparatus, MA). Isolated rat hearts were perfused for 60 min and then subjected to 15 min global ischemia followed by 30 min reperfusion to simulate the ischemic and reperfusion phase of MI/R injury. The rapid release of PKC βII within 5 min of reperfusion significantly improved contractile function and reduced infarct size compared to control I/R. Therefore, PKC βII inhibitor would be an effective tool to evaluate the role of PKC βII in the process of MI/R.

Results
All data in the text and figures are presented as means ± standard error of the mean (SEM). *p < 0.05 was considered significant compared to control I/R. **p < 0.01 was considered statistically significant compared to control I/R. Values of *p < 0.05 are statistically significant.

Statistical Analysis
To evaluate the role of PKC βII in the process of MI/R.

Conclusions
Reperfusion injury following myocardial ischemia has been shown to be a pathologic condition resulting in myocardial cell death and contractile dysfunction. PKC βII peptide inhibitor given at the beginning of reperfusion significantly improved contractile function and decreased infarct size compared to I/R control at 45 and 90 min post-reperfusion following 30 min global ischemia. These data suggest that PKC βII inhibition attenuates I/R-induced heart injury and thereby salvages heart tissue and function when given during reperfusion. These effects may be related to inhibiting ROS release in MI/R. Therefore, PKC βII inhibitor will be an effective therapeutic tool to ameliorate cardiac contractile dysfunction and tissue damage in heart attack, coronary bypass, and organ transplant patients.

References