Introduction

Coronary heart disease is the leading cause of death worldwide, and is primarily attributable to the detrimental 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 pharmacological 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 and known to increase infarct size [4,5]. PKC βII is known to increase NADPH oxidase activity in leukocytes, endothelial cells and cardiac myocytes via phox47 phosphorylation, and decrease eNOS activity via phosphorylation of eNOS [4,5,7,8]. NADPH oxidase produces superoxide (O2•−) and quenches endothelial derived NO in cardiac endothelial cells. Moreover, PKC βII phosphorylation of phosphatidic acid 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 pharmacological 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 50 min, in isolated perfused rat hearts followed by 45 or 90 min reperfusion. A cell permeable PKC βII peptide inhibitor (10-20 µM) was given at the beginning of reperfusion for five minutes. Post-reperfusion cardiac function and infarct size were measured and compared to untreated control I/R hearts. In addition, the use of PKC βII peptide inhibitor (10-20 µM) correlated with the inhibition of SO release from isolated leukocytes (poster P 168). These findings suggest that PKC βII activation contributes to reperfusion injury and PKC βII peptide inhibitor may mitigate reperfusion injury by inhibiting ROS release.

Hypothesis

We hypothesize that PKC βII peptide inhibitor will improve postreperfusion cardiac function and reduce infarct size in isolated perfused rat hearts (ex vivo) subjected to global IR compared to non-drug control I/R hearts in both MI(30min)/R(45min) & MI(50min)/R(90min) studies.

Methods

Isolated Rat Heart Preparation

Male Sprague Dawley (SD) rats (275-325 g) were anesthetised i.p with 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 Kreb's buffer saturated with 95% O2-5% CO2 maintained at 37°C and pH 7.3-7.4 by Langendorff preparation. Hearts were subjected to 15 min of baseline perfusion. 30 min of ischemia, and a 45 min or 90 min reperfusion period. End of plasma (control I/R hearts), or plasma containing cell-permeable PKC βII peptide inhibitor (N-(6-Mercaptopurinyl)-N,3-diethyldithiocarbamate (EDC) (100 µM) was used to protect the hearts during reperfusion. A constant flow rate of 150 ml/min was used to perfuse the isolated rat hearts (n=9).

Results

All data in the text and figures are presented as means ± S.E.M. Analysis of variance using post hoc analysis with the Student-Newman-Keuls test was used for statistical analysis. LVDP in all treatments was expressed as the percentage of change from 100% (n=9) and heart rate was taken at the beginning of reperfusion significantly improved contractile function and infarct size in heart function (55±3% maximal or 90 min) hearts. This improvement was an effective technique to ameliorate cardiac dysfunction and tissue damage in heart attack, coronary bypass, and organ transplant patients.

Conclusion

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-perfusion following 30 min global ischemic injury. These data suggest that PKC βII inhibition attenuates IR-induced heart injury and thereby salvages heart tissue 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 dysfunction and tissue damage in heart attack, coronary bypass, and organ transplant patients.

References