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 detrimental effects of tissue infarct after an ischemic insult. The most effective therapeutic intervention 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 infarction 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 and known to exacerbate tissue injury [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 Thr 495 [6,7,8]. NADPH oxidase produces superoxide (SO) and quenches endothelial derived NO in cardiac endothelial cells. Moreover, PKC βII phosphorylation of p66Shc at Ser 36 leads to increased mitochondrial reactive active 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 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-reperfused cardiac function and infarct size were measured and compared to untreated control I/R hearts. We found that PKC βII peptide inhibitor treated I/R hearts exhibited significantly improved post-reperfused cardiac function and reduced infarct size compared to 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 # P168). These findings suggest that PKC βII activation contributes to reperfusion injury and PKC βII peptide inhibitor may mitigate reperfusion injury by inhibiting ROS release.

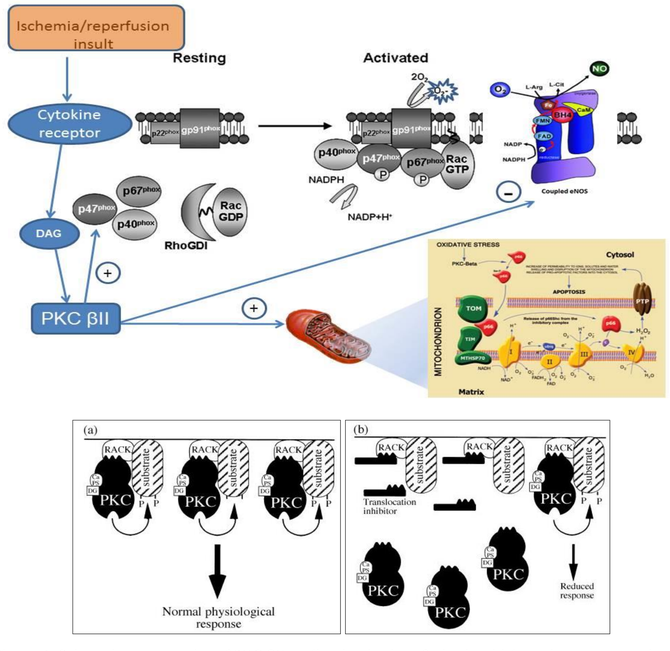

Figure 1. Schematic representation of PKC βII mediated activation of mitochondrial reactive oxygen species (ROS) and NADPH superoxide (O_2-) release and decreased NO release from eNOS in MI/R (top; adapted from [9]) and mechanism of action of PKC βII inhibitor (PKC βII-) (bottom; adapted from [10]). I/R induces cytokine receptor activation within minutes leading to activation of PKC βII via diacylglycerol (DAG) and increases ROS and O_2- release from damaged mitochondria and NADPH oxidase, respectively, and reduces eNOS activity. Activated PKC βII augments ROS release from mitochondria and decreases coupled eNOS activity to promote tissue injury during reperfusion (top). PKC βII- mechanism of action (bottom) is to inhibit PKC βII translocation to cellular substrates such as eNOS, NADPH oxidase, and mitochondrial p66Shc protein that increase ROS leading to opening of the mitochondrial permeability transition pore (PTP) which in turn leads to consequent release of proapoptotic factors into the cytosol. Therefore, inhibiting PKC βII would attenuate the aforementioned processes in I/R injury.

Hypothesis

We hypothesize that PKC βII peptide inhibitor will improve postreperfused cardiac function and reduce infarct size in isolated perfused rat hearts (*ex* vivo) subjected to global I/R compared to non-drug control I/R hearts in both MI(30min)/R(45min) & MI(30min)/R(90min) 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% O_2-5% CO_2 maintained at 37ºC and pH 7.3-7.4 by Langendorff preparation. Hearts were subjected to 15min of baseline perfusion, 30min of ischemia, and a 45min or 90 min reperfusion period. 5ml of plasma (control I/R hearts), or plasma containing cell-permeable PKC βII peptide inhibitor (N-Myr-SLNPEWNET, MW=1300 10 - 20μM Genemed Synthesis Inc. San Antonio TX) were infused during the first 5min of reperfusion by a side arm line proximal to the heart inflow at a rate of 1ml/min. Coronary flow, left ventricular developed pressure (LVDP), maximal and minimal rate of LVDP ($+dP/dt_{max}$ and $-dP/dt_{min}$), and heart rate were taken every 5min during baseline and reperfusion using a flow meter (T106, Transonic Systems, Inc., Ithaca, NY) and pressure transducer (SPR-524, Millar Instruments, Inc., Houston, TX), respectively. Data were recorded using a Powerlab Station acquisition system (ADInstruments, Grand Junction, CO). Sham hearts received no drug and were perfused with plasma at the same time point as I/R hearts and experienced no ischemia. To evaluate tissue viability, the left ventricle was isolated and cross sectioned into five 2mm pieces from apex to base at the end of the cardiac function experiment. The pieces were subjected to 1% triphenyltetrazolium chloride (TTC) staining for 15min at 37ºC to detect infarct size (viable tissue stained red, infarct left unstained (white)). Infarct size was expressed as the percentage of dead tissue to the total tissue weight.

Statistical Analysis

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 heart function and infarct size in the MI(30min)/R(90min) study. Student's t-test was used to compared the two groups in the MI(30min)/R(45min) study. Probability values of <0.05 are statistically significant.

Results

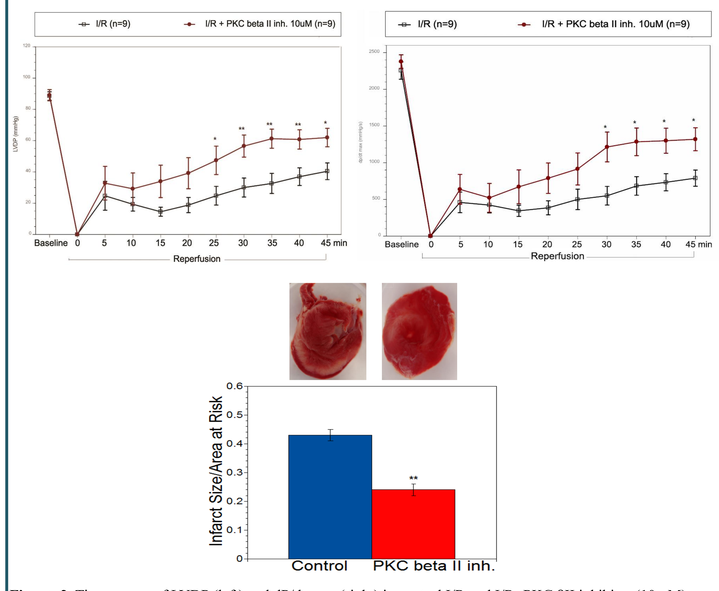

Figure 2. Time course of LVDP (left) and dP/dt max (right) in control I/R and I/R+PKC βII inhibitor (10 μM) perfused rat hearts. LVDP and dP/dt max data at initial (baseline) and reperfusion from 0 to 45 min following 30 min ischemia are shown. I/R+PKC βII inhibitor hearts (n=9) exhibited a significant improvement in postreperfused (45min) LVDP (70±6% of initial) and dP/dt max (55±6% of initial) compared to control I/R hearts (n=9). Representative TTC stained heart sections displayed above from control I/R and I/R+PKC βII inhibitor hearts were assessed after cardiac function experiments to determine infarct size. Viable tissue stained red and infarcted tissue was unstained (white). PKC βII inhibitor hearts displayed a significantly reduced infarct size (24±3%) compared to control I/R hearts (43±2%). All values are expressed as mean ± SEM. *p<0.05 and **p<0.01 from I/R control.

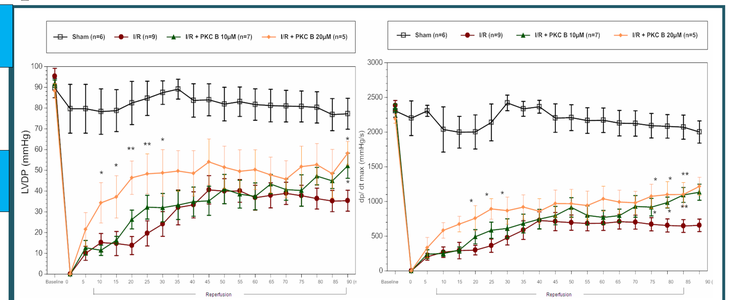

Figure 3. Time course of LVDP (left) and dP/dt max (right) in sham, control I/R and I/R+PKC βII inhibitor (10 and 20 μM) perfused rat hearts. LVDP and dP/dt max data at initial (baseline) and reperfusion from 0 to 90 min following 30 min ischemia are shown. I/R+PKC βII inhibitor hearts (10 μM, n=7; 20 μM, n=5) exhibited a significant improvement in LVDP 66 ± 8% and dP/dt max 56 ± 8% (20 μM) and 57±7% and 48±5% (10 μM) compared to control I/R hearts (n=9) that only recovered to 38±6% and 28 ±4% at 90 min post-reperfusion of initial baseline. Sham hearts maintained cardiac function throughout the experimental protocol (87±9% of initial LVDP and 89±8% of initial dP/dt max). (*p<0.05; **p<0.01 compared to control).

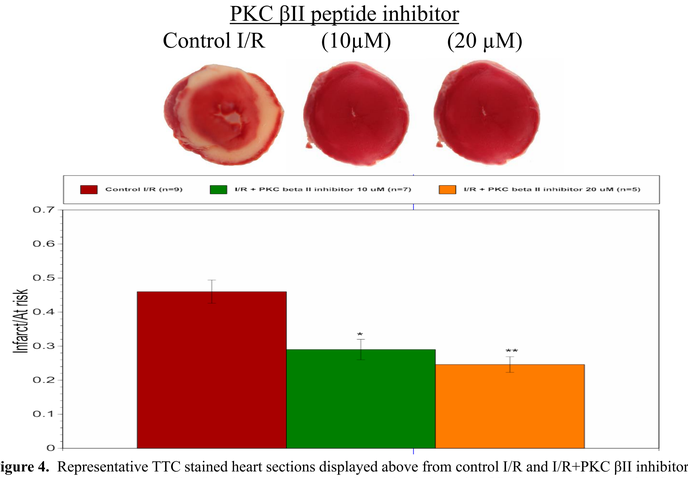

Figure 4. Representative TTC stained heart sections displayed above from control I/R and I/R+PKC βII inhibitor hearts were assessed after cardiac function experiments to determine infarct size. Viable tissue stained red and infarcted tissue was unstained (white). PKC βII inhibitor hearts displayed a significantly reduced infarct size (29 ±3%, 10 uM; 25 ±3%, 20 uM) and compared to control I/R hearts that had an infarct size of 46 ±3,% . Sham hearts had minimal cell death (<0.05%) at the end of the experimental protocol (data not shown). (*p<0.05 and **p<0.01 compared to I/R control).

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 salvage 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

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