Protein Kinase C Beta II Peptide Inhibitor Elicits Robust Effects on Attenuating Myocardial Ischemia/Reperfusion Injury

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Introduction

Heart disease remains the leading cause of death in adults in the United States and worldwide, with coronary artery disease being the most common form that often leads to a myocardial infarction. While rapid restoration of coronary blood flow is crucial to preserving cardiac tissue function, it also results in an additional insult known as myocardial ischemia/reperfusion (MI/R) injury. MI/R injury may be attenuated by inhibiting the generation of reactive oxygen species (ROS) upon cardio-angioplasty following a heart attack. Protein kinase C beta II (PKCβII) generates ROS during reperfusion via cytokine receptor activation (Figure 1) (1). Activated PKCβII (via Ca^{2+} and second messenger diacylgylcerol) binds to its selective receptor for activated C kinase (RACK). RACK enhances PKCβII translocation to the cell membrane and its interaction with substrates, like NADPH oxidase (NOX-2) (2). PKCβII phosphorylates and activates NOX-2 which then generates ROS (Figure 2) (3,4).

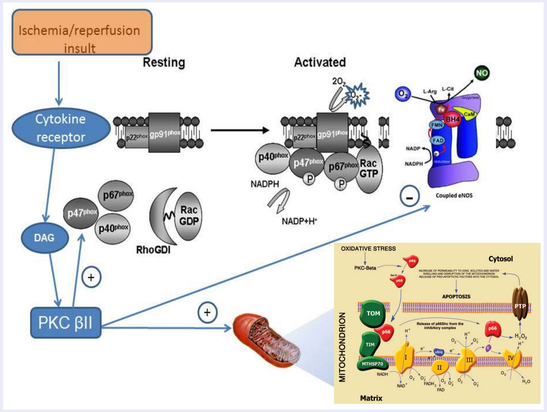


Figure 1. . Schematic representation of PKCβII mediated activation of mitochondrial reactive oxygen species (ROS) and NADPH oxidase superoxide (O_2-) release and decreased NO release from eNOS in MI/R (adapted from [5,6]). MI/R induces cytokine receptor activation leading to activation of PKC βII via diacylglycerol (DAG). Activated PKCβII increases ROS and O_2- release from damaged mitochondria and NADPH oxidase, respectively, and decreases eNOS activity. It also stimulates mitochondrial p66Shc protein, a component in the pathway resulting in opening of the mitochondrial permeability transition pore (PTP), which in turn leads to release of proapoptotic factors into the cytosol to further promote tissue injury during reperfusion.

Inhibition of tissue NOX-2 attenuates inflammation mediated vascular injury seen in various diseases, including diabetes and myocardial infarction (4). Previously, a myristoylated (myr-) selective PKCβII peptide inhibitor (*N*-myr-SLNPEWNET; myr-PKCβII-) was found to dose dependently inhibit superoxide (SO) release and MI/R injury via the mechanism depicted in Figure 2 (3, 6, 7). Myristoylation of peptides is known to potentiate their entry into the cell via simple diffusion through the cell membrane to affect PKC activity (8). However, the effect of myr-PKCβII peptide activator (*N*-myr-SVEIWD; myr-PKCβII+) on MI/R injury has not been studied (9).

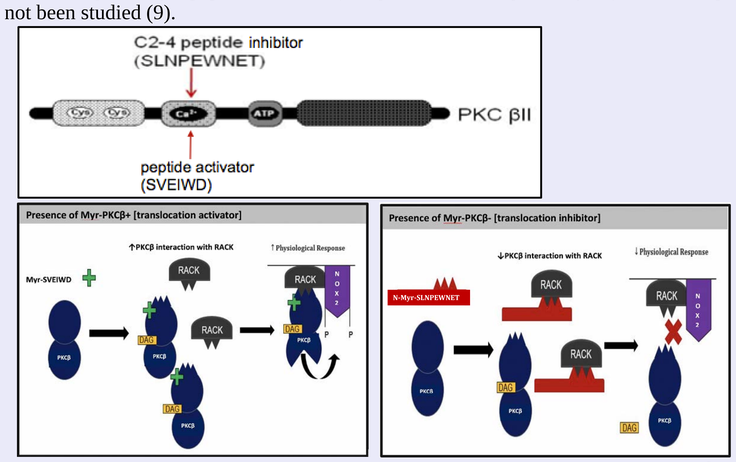


Figure 2. Schematic representation of PKCβII peptides. PKCβII+ and PKCβII- both bind to the Ca^{2+} binding domain within the RACK binding site (i.e., C2-4 region;) of PKCβII to regulate its translocation to the cell membrane (top; Adapted from [3]). PKCβII+ mechanism of action is to increase PKCβII translocation to the cell membrane via RACK binding and its interaction with substrates, like NOX-2, while PKCβII- inhibits that interaction (bottom; Adapted from [2]).

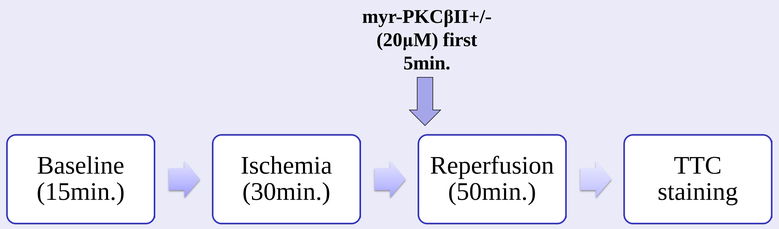
Hypothesis

Of the many proteins that PKCβII phosphorylates, for the purposes of this study, we believe NOX-2 phosphorylation is a key pathway in the ROS mediated damage in MI/R injury. Thus, we hypothesize that myr-PKCβII- will reduce infarct size and improve post-reperfused cardiac function as compared to non-drug treated controls, whereas myr-PKCβII+ treated hearts will not improve these parameters.

Research Design

Male Sprague-Dawley rats (~300g, Charles River, Springfield, MA) were anesthetized with I.P. pentobarbital (60mg/kg) and anticoagulated with 1000U of heparin. The heart was then removed and placed on the perfusion needle of the Langendorff apparatus. A pressure transducer was placed into the left ventricle to measure cardiac function, as previously described (6,7).

A schematic of the MI/R protocol is depicted below:



At the end of the reperfusion period, all hearts were frozen at -20 ºC for 30 min, sectioned into 2mm slices and incubated at 37ºC in 1% triphenyltetrazolium chloride (TTC). The percentage between dead heart tissue (i.e., unstained) weight to total heart tissue weight was calculated for infarct size.

Statistical Analysis

All data in the text, figures, and table are presented as means ± S.E.M. The data were analyzed by ANOVA using the Fisher's PLSD test. Probability values of <0.05 are considered to be statistically significant.

Results

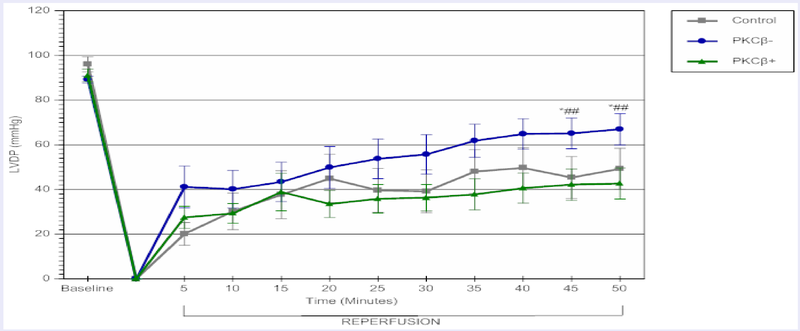
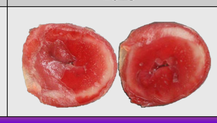
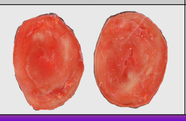
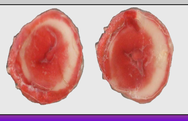


Figure 3. Time course of left ventricular developed pressure (LVDP) for control, myr-PKCβII+/- MI/R studies. Myr-PKCβII- treated hearts showed a trend to improve LVDP after the first five minutes of reperfusion and this trend was exhibited throughout the 50 minute reperfusion time course. This was significantly different from both control and myr-PKCβII+ treated hearts during the reperfusion period. *p<0.05 vs. non-drug treated controls, ##p<0.01 vs. myr-PKCβII+.

Table 1. Initial and final cardiac function values and infarct size for control, myr-PKCβII+/- MI/R studies; *p<0.05, **p<0.01 vs. non-drug treated controls; #p<0.05, ##p<0.01 vs. myr-PKCβII+. Representative sections shown are both sides of a 2mm mid-wall section for each MI/R study group. $+dP/dt_{max}$ = contractility, $-dP/dt_{min}$ = relaxation, LVDP = left ventricular developed pressure, LVESP = left ventricular end systolic pressure, LVEDP = left ventricular end diastolic pressure.

Cardiac Function and Infarct Size Indices	Control (n= 9)	PKCβ Inhibitor (n=8)	PKCβ Activator (n=9)
Initial Flow (mL/min)	20±2	17±1	18±2
Final Flow (mL/min)	10±1	10±1	11±2
Initial $+dP/dt_{max}$ (mmHg/sec)	2428±81	2316±48	2430±65
Final $+dP/dt_{max}$ (mmHg/sec)	906±137	1585±165**##	989±161
Initial $-dP/dt_{min}$ (mmHg/sec)	-1685±87	-1637±46	-1630±76
Final $-dP/dt_{min}$ (mmHg/sec)	-855±118	-1048±121*#	-814±103
Initial LVDP (mmHg)	96±3	89±2	91±3
Final LVDP (mmHg)	48±9	67±7*##	43±7
Initial LVESP (mmHg)	105±4	98±2	98±3
Final LVESP (mmHg)	106±5	104±5	104±3
Initial LVEDP (mmHg)	9±1	8±1	7±1
Final LVEDP (mmHg)	58±5	37±7**##	58±4
Initial Heart Rate (BPM)	273±5	276±8	280±10
Final Heart Rate (BPM)	258±8	247±5	246±8
Infarct Size (%)	26±5	14±3*	25±3
Representative Sections			

Conclusions

Infarct size

Myr-PKCβII- treated hearts had significantly reduced infarct size compared to controls. We believe there was no significant difference between myr-PKCβII- and myr-PKCβII+ because NOX-2 mediated ROS generation during reperfusion may be maximally activated by tissue cytokines and further stimulation by myr-PKCβII+ does not result in additional tissue injury.

Cardiac function

Myr-PKCβII- improved post-reperfused cardiac function (vs. both control and myr-PKCβII+). The significant improvement in final post-reperfusion LVDP in myr-PKCβII- treated hearts is attributed to the significant reduction in final LVEDP values (i.e. ~37mmHg) compared to control and myr-PKCβII+ hearts (i.e. ~58mmHg), and is reflected in the significant restoration of the final maximal rate of contractility ($+dP/dt_{max}$) and relaxation ($-dP/dt_{min}$).

These results suggest that: 1) Inhibition of myocardial tissue NOX-2 activity may be the principal pathway through which myr-PKCβII- mediates its cardio-protective effects in MI/R injury. **2)** Treatment with myr-PKCβII- would be an effective strategy to limit MI/R injury in heart attack patients upon reperfusion via fibrinolytic therapy, angioplasty or coronary artery bypass surgery.

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