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Myristoylated Protein Kinase C Epsilon Peptide Inhibitor Exerts Cardioprotective Effects in Rat and Porcine Myocardial Ischemia/Reperfusion: A Translational Research Study

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Introduction

Following an acute myocardial infarction, the rapid restoration of blood flow to the ischemic myocardium is the most effective method of limiting infarct size and improving patient outcomes. However, reperfusion leads to the loss of cardiomyocytes that were viable during the ischemic episode and is referred to as ischemia/reperfusion (I/R) injury [1]. This restoration of blood flow is known to result in the overproduction of reactive oxygen species (ROS), as incoming oxygen reacts with the damaged mitochondrial respiratory chain to produce superoxide that in turn leads to the enzymatic uncoupling of endothelial nitric oxide synthase (eNOS) and the production of more ROS and reduced nitric oxide (NO) bioavailability [2]. The reduction in NO has been shown to reduce the coupling efficiency of O_2 consumption and ATP synthesis in cardiac mitochondria, presumably leading to further ROS production during in I/R [3]. It is well established that protein kinase C epsilon (PKCε) activity can stimulate eNOS activity. During I/R, PKCε activity is activated via stimulation of cytokine receptors and enhances uncoupled eNOS activity [4]. We hypothesized that limiting PKCε activity during I/R using a cell permeable myristoylated PKCε peptide inhibitor (PKCε-) should limit ROS production in part by attenuating uncoupled eNOS activity and thereby improve heart function and reduce infarct size.

Results and Discussion

Untreated isolated rat (male Sprague-Dawley, 275-325g) hearts subjected to global I(30min)/R(45min) exhibited compromised cardiac function, while I/R hearts treated with 10μM PKCε- (N-myr-EAVSLKPT, MW=1054g/mol, Genemed Synthesis) given at the beginning of reperfusion had significant restoration for all cardiac function indices and coronary flow (Figure 1). Infarct size was significantly ($p < 0.01$) reduced from 48±2 (n=10) to 25±2% (n=11) in untreated compared to treated hearts (Figure 3). We also studied regional myocardial I(1hr)/R(3hr) injury in anesthetized pigs (castrated male Yorkshire Cross, 27-36kg) in which the left anterior descending coronary artery was occluded at the level of the second diagonal branch for 1hr using a fluoroscopically guided balloon catheter. Echocardiography was used to monitor ejection fraction during baseline, ischemia and post-reperfusion (1-3hr). PKCε- (0.8mg/kg, n=3) was given at reperfusion. At the end of the reperfusion period, cardiac ejection fraction in hearts treated with PKCε- recovered to 91±6% (n=3) of its baseline value (Figure 2), and infarct size was 13±0.3% (n=3) of the total area at risk (Figure 3). These parameters were significantly improved compared to saline treated myocardial I/R pigs that recovered to a final cardiac ejection fraction of only 70±3% ($n = 4$, $p<0.01$) of baseline values and an infarct size of 34±4% ($n = 3$, $p<0.01$; Figures 2 and 3).

These results suggest that myristoylated PKCε- has profound cardioprotective effects that transcend species in both global (rat) and regional (porcine) I/R, which may be mediated by the inhibition of ROS production from uncoupled eNOS activity. Collectively these data suggest that PKCε- should be an effective therapeutic tool to ameliorate cardiac contractile dysfunction and tissue damage following acute myocardial infarction and subsequent primary coronary intervention treatment in humans.

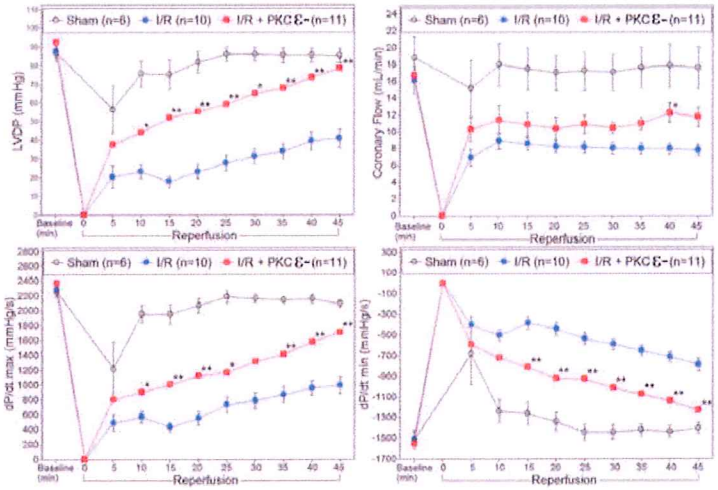

*Fig. 1. Time course of left ventricular developed pressure (LVDP) (top left), coronary flow (top right), dP/dtmax (bottom left), and dP/dtmin (bottom right) in isolated perfused rat hearts subjected to 30 min ischemia prior to reperfusion. (*p<0.05,**p<0.01 vs. I/R control). Data were analyzed using ANOVA with the Student-Newman-Keuls test.*

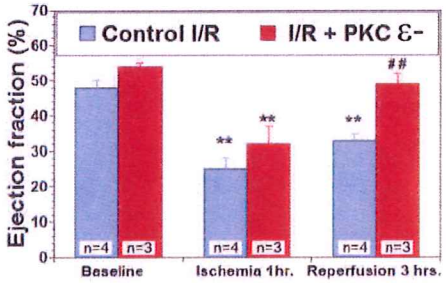

*Fig 2. Ejection fraction of saline control and PKCε-treated I/R hearts during baseline, ischemia and 3hr reperfusion. Both groups exhibited a significant decrease in ejection fraction during ischemia compared to their respective baselines (**p<0.01 vs. hearts at baseline). Ejection fraction for PKCε-treated hearts was significantly increased compared to control hearts at 3hr reperfusion ($^{\#\#}p<0.01$).*

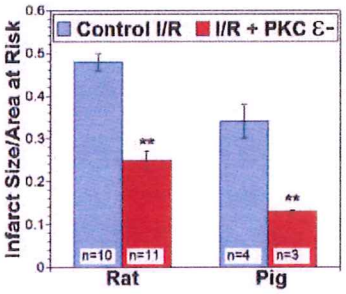

*Fig 3. Ratio of infarcted tissue weight to that of area at risk. PKCε- treatment significantly decreased infarct size compared to untreated control I/R rat hearts (** p<0.01). PKCε-treated pig hearts had an infarct size of 13±0.3% , which was significantly reduced compared to saline control pigs with an infarct size of 34±4% (**p<0.01).*

Acknowledgments

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References

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