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Protein Kinase C Epsilon Peptide Inhibitor Exerts Cardioprotective Effects in Myocardial Ischemia/Reperfusion Injury

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Abstract

Background: The generation of reactive oxygen species (ROS) during myocardial ischemia (I)/reperfusion (R) contributes to post-reperfusion cardiac injury. The increase in ROS is attributed in part to the uncoupling of endothelial nitric oxide synthase (eNOS) that produces ROS instead of nitric oxide (NO) and mitochondrial derived ROS which in part is derived from opening of mitochondrial ATP-sensitive K^+ channels (mK_{ATP}). Both the activity of uncoupled eNOS and the opening of mK_{ATP} channels in mitochondria are stimulated by protein kinase C epsilon (PKCε) that is activated during reperfusion. We hypothesize that a cell permeable PKCε peptide inhibitor, (N-myristic acid-EAVSLKPT, Myr-PKCε-) given at reperfusion will improve post-reperfused cardiac function and attenuate infarct size compared to untreated isolated perfused rat hearts subjected to ischemic reperfusion injury.

Methods: Male Sprague-Dawley rats (275-325 g) were anesthetized with sodium pentobarbital (60 mg/kg) and anticoagulated with heparin 1000 units given intraperitoneally. The hearts were excised and attached to a Langendorff perfusion system. All hearts were subjected to 30 min of global ischemia, followed by 90 min of reperfusion. Myr-PKCε- was dissolved in Krebs' buffer and infused during the first 10 min of reperfusion at final concentrations of 5, 10 and 20 μM.

Results: Myr-PKCε- treated hearts (10 and 20 μM) exhibited significant improvement in post-reperfused cardiac function at 90 min compared to untreated controls. The maximal rate of left ventricular developed pressure generation ($+dP/dt_{max}$) of Myr-PKCε- treated hearts showed significant recovery to 56±4% (10 μM, $p<0.05$, n=8) and 50±3%; (20 μM, $p<0.05$, n=8) of initial baseline values. By contrast, the $+dP/dt_{max}$ of both untreated control hearts (n=9) and those treated with the lowest concentration of Myr-PKCε- (5 μM, n=8) recovered to only 30±4% and 33±4% of initial baseline values, respectively. Interestingly, all Myr-PKCε- treated hearts (5-20 μM) showed significant reduction in infarct size to 28±2% compared to untreated control hearts, which averaged 38±3% ($p<0.05$, n=9).

Conclusion: The results suggest that Myr-PKCε- given at reperfusion effectively reduces infarct size, improves cardiac function and is a putative treatment that could aid in clinical myocardial infarction/organ transplantation patient recovery.

Introduction

Myocardial cell death from ischemia-reperfusion (I/R) injury (e.g. myocardial infarction) continues to be a major cause of morbidity and mortality in Western nations [1]. Reperfusion is essential to salvage ischemic myocardial tissue from infarction. However, the re-establishment of blood flow to acutely ischemic myocardium leads to rapid cell death and compromised heart function [2]. The size of the infarct that results from the combination of the ischemic and reperfusion injury is the major determinant of the prognosis of patients who survive the acute myocardial infarction incident [3-7]. The necessity for effective pharmacological intervention still exists, despite numerous basic studies firmly establishing that I/R injury can be delayed or even reduced by the introduction of cardioprotective agents or strategies prior to a prolonged ischemic insult (preconditioning) or at the start of reperfusion [1,8]. Such interventions have been shown to reduce infarct size, attenuate the frequency and severity of reperfusion-induced arrhythmias, and prevent endothelial dysfunction [9-13].

Preconditioning of the myocardium can be induced by several rounds of brief ischemia (i.e. less than 4 min) followed by several rounds of brief reperfusion (i.e. less than 6 min) prior to prolonged ischemia (i.e. 30 min or greater) followed by the final reperfusion period [12]. The precise mechanism for ischemic preconditioning remains unclear; however, there is growing evidence that activation of protein kinase C epsilon (PKCε) plays a central role [12,14-16]. The transient ischemic episodes are thought to activate PKCε directly by cytokine induced production of intracellular diacylglycerol (DAG) and indirectly by eliciting a small amount of reactive oxygen species (ROS) release from the transient opening of the mitochondrial ATP-sensitive K^+ channels (mK_{ATP}) on the inner mitochondrial membrane. ROS activates PKCε via oxidative modification of its regulatory domain. Activated PKCε translocates to the mK_{ATP} channel releasing additional ROS which in turn further activates PKCε in a positive feedback loop [17-19]. The resulting ROS then activates a second PKCε mitochondrial pool that prevents the opening of the mitochondrial permeability transition pore (MPTP). The MPTP is a large conductance channel in the inner mitochondrial membrane whose extended opening leads to the dissipation of the inner mitochondrial membrane potential, matrix swelling and rupture of the outer mitochondrial membrane, ultimately leading to necrosis [20-24].

Results from several studies generally support the role of PKCε activation in preconditioning, for example, agents that mimic preconditioning also activate PKCε such as adenosine, volatile anesthetics, and exogenously applied PKCε agonists [16,25-28].

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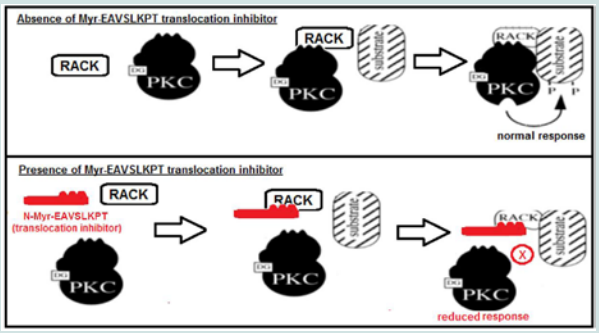

Figure 1: Mechanism of action of myristoylated (Myr)-PKCε- (shown in red, adapted from [36]). PKCε when activated by diacylglycerol (DG) binds to a specific receptor for activated C kinase (RACK) domain which translocates PKCε to interact with substrates (e.g. uncoupled eNOS) to produce a physiologic response (top panel). Myr-PKCε- inhibits PKCε translocation by binding to RACK to reduce the physiologic response evoked by PKCε phosphorylation of its target substrate (bottom panel).

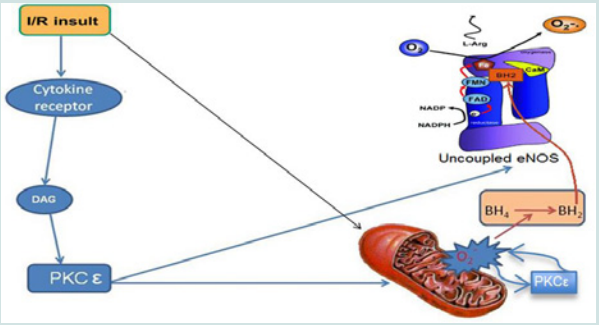

Figure 2: Schematic representation of PKCε regulation of mitochondrial and uncoupled eNOS superoxide (O_2-.) release in myocardial I/R (adapted from [37]). I/R insult activates PKC (e.g. PKCε) and causes overproduction of O_2-. from damaged mitochondria, thereby leading to oxidation of tetrahydrobiopterin (BH_4) to dihydrobiopterin (BH_2). BH_2 promotes eNOS uncoupling which produces O_2-. instead of NO resulting in additional I/R induced oxidative stress. Activated PKCε phosphorylation increases uncoupled eNOS activity and opens mitochondrial ATP-dependent potassium channels during reperfusion leading to increased O_2-. induced exacerbation of I/R injury [34,38].

Moreover, preconditioning of isolated hearts from PKCε knockout mice failed to reduce I/R injury [12]. Conversely, in studies in which PKCε activity was promoted using a PKCε peptide activator (HDAPIGYD) given during reperfusion rather than prior to ischemia, no cardioprotective effects were exhibited [13,29].

Our research group confirmed these findings and discovered that a cell permeable myristic acid conjugated PKCε peptide inhibitor (Myr-PKCε-) dramatically attenuated cardiac contractile dysfunction in leukocyte-augmented myocardial I/R injury when given during reperfusion [13]. Myr-PKCε- acts as a competitive translocation inhibitor by mimicking the receptor for activated C kinase (RACK) binding site on PKCε (see Figure 1) [30]. During reperfusion in this I/R model, three principle sources of ROS will be reduced directly and indirectly by inhibition of PKCε activity, opening of the mK_{ATP} channels, uncoupled endothelial nitric oxide synthase (eNOS) activity, a switch from nitric oxide (NO) to ROS production, and intracellular adhesion molecule-1 (ICAM-1) mediated leukocyte ROS release (see Figure 2) [31-35]. We previously reported that PKCε activation upon reperfusion is not cardioprotective, unless eNOS uncoupling is reversed to its normal or coupled state by the addition of an essential cofactor, tetrahydrobiopterin (BH_4) [34]. During reperfusion, eNOS uncoupling is promoted by BH_4 oxidation to dihydrobiopterin (BH_2). Since BH_2 and BH_4 have equal affinity for the eNOS oxygenase-binding domain, an increase in the BH_2 to BH_4 ratio during reperfusion shifts eNOS towards the uncoupled state [34]. Hence, inhibiting PKCε during reperfusion will attenuate ROS mediated insults arising from uncoupled eNOS activity, the mK_{ATP} channel, and leukocyte endothelial adhesion and penetration.

We previously found that Myr-PKCε- (10 μM) given at reperfusion elicited cardioprotective effects in an isolated perfused rat heart model subjected to I (30 min)/R (45 min) [39]. The aim of the present study is to determine the optimal concentration for the cardioprotective effects of Myr-PKCε- when the reperfusion period is doubled to 90 min.

Methods

Concentration-dependent cardioprotective effects of Myr-PKCε- (5, 10 and 20 μM) were evaluated based on improved cardiac function and reduction of infarction size compared to untreated (control) I/R hearts.

Male Sprague-Dawley rats (275-325 g, Charles River, Springfield, MA) were anesthetized via an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and anticoagulated with heparin 1000 units. Hearts were quickly isolated and attached to a Langendorff apparatus as previously described [39]. The I/R protocol consisted of a 15 min stabilization period, 30 min of global ischemia, and 90 min of reperfusion. The following cardiac function parameters were measured throughout the entire protocol using a data acquisition system (PowerLab/8Sp, ADInstruments, Colorado Springs, CO). Heart rate, left ventricular end systolic pressure (LVESP), end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP, i.e. LVESP-LVEDP), and the maximal rate of left ventricular pressure generation ($+dP/dt_{max}$) and decline ($-dP/dt_{min}$) were measured using a pressure transducer (SPR-524; Millar Instruments, Inc., Houston, TX) placed in the left ventricle. Coronary flow was monitored by a flowmeter (T106; Transonic Systems, Inc., Ithaca, NY). A stock of Myr-PKCε- (N-Myr-EAVSLKPT; MW=1054 g/mol; (Genemed Synthesis, Inc., San Antonio, TX) was prepared by solubilizing the appropriate amount of the compound in 28% dimethyl sulfoxide (DMSO). This stock solution was further diluted to yield a final concentration of 5, 10 or 20 μM in 0.03% DMSO in the perfusate. This compound was then infused during the first 10 min of reperfusion at a rate of 1 ml/min via a syringe pump.

To determine infarct size, all hearts were removed from the apparatus, lightly blotted, and placed in a -20 ˚C freezer for 30 min. The partially frozen heart was sectioned perpendicular to its long axis into 2 mm thick slices. The slices were then incubated at 37 ˚C in a 0.2 M Tris buffer (pH=7.41) containing 1% 2,3,5-triphenyltetrazolium chloride (TTC) for ~5 min to demarcate the infarcted (white) from the viable (red) tissue. Stained heart slices were transferred to 4% paraformaldehyde solution for ~15 min to improve the contrast between the infarcted and viable tissue areas. The infarct size was

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Table 1: Cardiac function initial (baseline) and final values for control I/R and I/R+Myr-PKCε- (5, 10 and 20 μM) treated hearts. LVESP, left ventricular end-systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVDP, left ventricular developed pressure; maximal rate of LV pressure generation (+dP/dt$_{max}$) and decline (-dP/dt$_{min}$). Data expressed as mean±S.E.M.*p < 0.05; **p < 0.01 vs. control I/R. #p < 0.05; ##p < 0.01 vs. I/R + Myr-PKCε- (5 μM).

	Control I/R (n=9)	I/R + PKCε- 5μM (n=8)	I/R + PKCε- 10μM (n=8)	I/R + PKCε- 20μM (n=8)
Initial LVESP (mmHg)	102.1 ± 1.8	97.1 ± 3.1	99.2 ± 4.2	102.3 ± 3.6
Initial LVEDP (mmHg)	9.5 ± 0.83	7.4 ± 0.76	9.0 ± 0.32	9.0 ± 0.97
Initial LVDP (mmHg)	92.5 ± 1.6	89.7 ± 3.1	90.2 ± 4.2	93.4 ± 3.0
Final LVESP (mmHg)	95.4 ± 3.1	87.2 ± 6.7	106.2 ± 3.1	107.7 ± 6.5
Final LVEDP (mmHg)	58.5 ± 5.5	46.2 ± 7.8	46.4 ± 4.7	57.6 ± 6.3
Final LVDP (mmHg)	36.8 ± 4.0	41.0 ± 4.2	59.8 ± 3.5## **	50.1 ± 2.3*
Initial +dP/dt$_{max}$ (mmHg/s)	2346 ± 70	2308 ± 80	2371 ± 65	2381 ± 88
Final +dP/dt$_{max}$ (mmHg/s)	714 ± 92	770 ± 104	1316 ± 94## **	1202 ± 63## **
Initial -dP/dt$_{min}$ (mmHg/s)	-1572 ± 62	-1543 ± 63	-1628 ± 79	-1595 ± 70
Final -dP/dt$_{min}$ (mmHg/s)	-525 ± 55	-546 ± 44	-874 ± 79## **	-741 ± 33#*
Initial Coronary Flow (mL/min)	17.2 ± 2.1	19.4 ± 1.1	20.6 ± 3.3	19.7 ± 1.6
Final Coronary Flow (mL/min)	7.6 ± 0.6	7.9 ± 1.2	7.7 ± 0.97	8.4 ± 1.3
Initial Heart Rate (BPM)	258.8 ± 15.3	271.4 ± 6.1	273.5 ± 20.9	287.6 ± 8.8
Final Heart Rate (BPM)	241.3 ± 10.7	243.9 ± 5.2	228.0 ± 21.4	242.5 ± 13.1

determined by carefully dissecting (under magnification) and weighing the corresponding tissue in both areas. The infarcted tissue weight was divided by total weight to yield percentage of infarcted tissue.

Statistical Analysis

All data are presented as the mean±S.E.M. ANOVA analysis using Student-Neuman-Keuls test was used to assess statistical difference in cardiac function parameters and infarct size between control (untreated) I/R and I/R+Myr-PKCε- (treated) hearts. Probability values of <0.05 were considered statistically significant.

Results

Hearts treated at reperfusion with 10 and 20 μM Myr-PKCε-, but not with 5 μM, exhibited improvement in the following parameters of cardiac function following 90 min of reperfusion: LVDP, and both dP/dtmax and min (see Table 1). The higher concentrations of Myr-PKCε- (10 and 20 μM) significantly restored +dP/dt$_{max}$ to 56±4% (p<0.05, n=8) and 50±3% (p<0.05, n=8) compared to treatment with lower concentrations of Myr-PKCε- (5 μM) and untreated control hearts which only recovered to 33±4% (n=8) and 30±4% (n=9) of initial baseline values respectively at the end of the reperfusion period (Figure 3).

Hearts treated with the lowest concentration of Myr-PKCε- (5 μM, n=8) recovered to 35±3% of baseline -dP/dt$_{min}$, which was not significantly different from untreated controls (Figure 4). While hearts

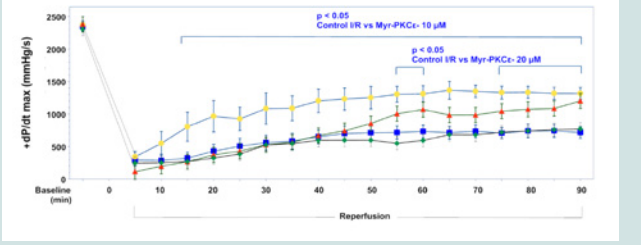

Figure 3: Time course of +dP/dt$_{max}$ for Control I/R (**blue**), I/R+Myr-PKCε- 5 μM (**green**), I/R+Myr-PKCε- 10 μM (**yellow**) and I/R+Myr-PKCε- 20 μM (**red**).

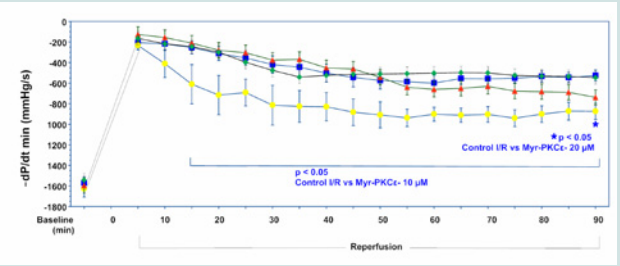

Figure 4: Time course of -dP/dtmin for Control I/R (blue), I/R+Myr-PKCε- 5 μM (green), I/R+Myr-PKCε- 10 μM (yellow) and I/R+Myr-PKCε- 20 μM (red).

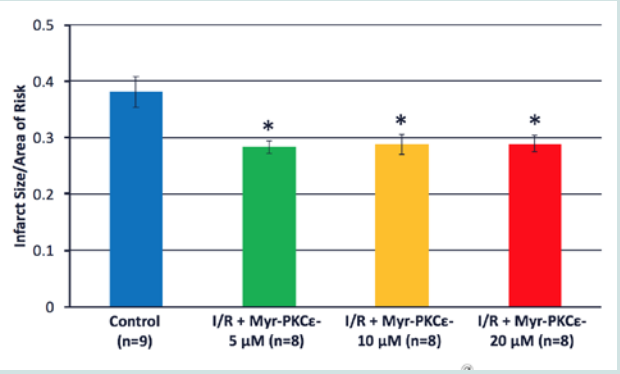

Figure 5: Percent infarcted size of control I/R (blue), I/R+Myr-PKCε- 5 μM (green), I/R+Myr-PKCε- 10 μM (yellow) and I/R+Myr-PKCε- 20 μM (red) after TTC staining.

*p<0.05 *vs.* control I/R hearts.

treated with higher concentrations of Myr-PKCε- showed significant restoration in left ventricular -dP/dt$_{min}$ at the end of the reperfusion period to 54±5% with 10 μM (p<0.05, n=8) and to 46±2% with 20 μM (p<0.05, n=8) of initial baseline values compared to untreated controls, which only recovered to 33±3% (n=9), respectively.

Hearts treated with higher concentrations of Myr-PKCε- (10 and 20 μM) exhibited significant improvement in post-reperfused LVDP.

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Recovery of LVDP reached 66±4% with 10 μM ($p<0.01$, n=8) and 54±3% with 20 μM ($p<0.05$, n=8) at 90 min of reperfusion compared to hearts treated with 5 μM (n=8) and control (n=9) hearts which only recovered to 46±5% and 40±4% of initial baseline values at the end of the reperfusion period (Table 1).

At all concentrations, Myr-PKCε- treated hearts showed significant reduction in infarct size, 28±2% (5 μM, n=8), 29±2% (10 μM, n=8) and 29±2% (20 μM, n=8), compared to untreated control I/R hearts, 38±3% ($p<0.05$, n=9) (Figure 5).

Discussion

Treatment with Myr-PKCε- at the onset of reperfusion was effective in restoring post-reperfused cardiac function at concentrations of 10 and 20 μM (see Figures 3 and 4) (Table 1) and reducing infarct size at all concentrations tested (5, 10 and 20 μM, see Figure 5). The time course of the recovery of cardiac function was slightly slower following 20 μM compared to 10 μM Myr-PKCε-. By contrast, 5 μM treated hearts showed no significant improvement in function throughout reperfusion compared to untreated control hearts. Nevertheless, infarct size in Myr-PKCε- treated hearts (5, 10 and 20 μM) was approximately the same: 28% (5 μM) and ~29% (10 and 20 μM). Yet, all treated hearts showed significant reduction in infarct size compared to controls (~38%, $p<0.05$). Interestingly, in a porcine acute intact regional I (1 hr)/R (3 hr) model, Montgomery M et al. showed that Myr-PKCε- given IV at reperfusion to yield an estimated circulating concentration of 10 μM (0.8 mg/kg) restored post-reperfused cardiac function (i.e. ejection fraction) by 91±3% of initial baseline values and reduced infarct size to 13±0.3% [39]. In comparison, untreated controls only recovered to 69±3% of initial baseline and had infarct sizes of 34±4%. Collectively, the results from these studies indicate that the cardioprotective effects of Myr-PKCε- are consistent across species and models of I/R injury.

It is unclear why the 5 μM treated hearts failed to show significant recovery in function despite a lower infarct size than control hearts. These results suggest that Myr-PKCε- effectively inhibits necrosis independently of function recovery, which may be due to continually elevated ROS levels in the low concentration treated hearts (i.e. 5 μM) that cause stunning. Previous myocardial I/R studies using a ROS-reducing antioxidant peptide targeted to the mitochondria have also reported a reduction in infarct size without improvement in contractility lasting days to weeks after reperfusion [40]. Moderately elevated levels of ROS could potentially contribute to stunning of reversibly damaged myocardial tissue while higher levels lead to necrosis. The lack of a concentration-dependent improvement in infarct size with increasing Myr-PKCε-concentrations indicates that the ED50 for this molecule on infarct size is markedly below 5 μM.

The absence of a concentration dependent effect of a cardioprotective agent on infarct size has been reported in other studies. Arakawa M et al. found no difference in infarct size in isolated perfused rat hearts treated with three concentrations (i.e. 5, 50, and 500 nM) of an anti-apoptotic drug (FNK) constructed from Bcl-x(L), a member of the Bcl-2 apoptotic regulator protein family [41]. In their study, all three concentrations tested resulted in a reduction in infarct size to ~30±5% (n=8) compared to 47±5% (n=8) in control hearts. Similar to our current study, the middle concentration tested (i.e. 50 nM) restored post-reperfused cardiac function (e.g. LVDP) more so than the higher and lower concentrations and was significantly different from controls. In another study of isolated perfused rat hearts, Abarbanell AM et al. reported no difference in infarct size between high (i.e. 1 μg) and low concentrations (i.e. 200 ng; ~38±2%, n=10) of a cytokine mediated inhibitor (HMGB1) given during reperfusion compared to controls (49±3%, n=9) despite differences in post-reperfused cardiac function (i.e. $+dP/dt_{max}$ and $-dP/dt_{min}$) [42]. The infarct size reduction reported in drug treated hearts (i.e. 23% of control) is similar to our results (i.e. 26% of control). Lastly, Zhang W et al. reported that aliskiren, a direct renin inhibitor, lowered blood pressure in a concentration dependent manner, whereas there was no difference in infarct size in rats subjected to regional cardiac I/R between the high (i.e. 60 mg/kg, lower blood pressure) and low concentrations (i.e. 30 mg/kg) of aliskiren [43]. Collectively, the results from these three studies targeting different cellular pathways are comparable to our current findings and suggest that there may be a finite efficaciousness to pharmacological interventions targeted at reducing infarct size when given during reperfusion. The primary mechanism that underlies this early lethal reperfusion injury is still unknown but likely includes Ca^{2+} dysregulation and changes in pH that occur during the ischemic phase. The influx of Ca^{2+} and the rapid restoration of physiologic pH during reperfusion leads to rapid dysfunction, hypercontracture and necrosis [44]. Thus deep reductions in infarct size during I/R injury may only be possible with preconditioning treatment that acts to limit and/or correct for this Ca^{2+} and pH dysregulation which begins during the ischemic phase [44].

The time course of recovery in function of $+dP/dt_{max}$ and $-dP/dt_{min}$ appeared to be optimal in the hearts treated with 10 μM Myr-PKCε-. By comparison, we previously showed that 10 μM concentration significantly restored cardiac function (i.e. $+dP/dt_{max}$) at 10 min reperfusion and maintained this effect throughout the reperfusion period whether it was 45 or 90 min reperfusion [39]. The results for the current study clearly shows that the 10 μM concentration PKCε- can maintain its cardioprotective effect for an additional 45 min without significantly altering infarct size [i.e. 25±2% (45 min reperfusion) *vs.* 29±2% (90 min reperfusion)] [39]. The relatively slow recovery of function in the 20 μM treated hearts suggests that this higher concentration may cause some direct stunning by the drug that in turn slows the rate of recovery. Similarly, Abarbanell AM et al. showed that higher concentration of a cytokine inhibitor did not result in improved cardiac function [42]. Interestingly, Koyanagi T et al. reported that PKCε- inhibits cytokine production after heart transplantation [45]. Therefore, it is possible that the higher drug concentrations may stun the myocardium slowing the rate of functional recovery. By contrast, hearts subjected to the lower concentration of Myr-PKCε- (5 μM) may reduce ROS production to a level that attenuates infarct size, but not sufficient to improve post-reperfused cardiac function (see LVDP, $+dP/dt_{max}$ and $-dP/dt_{min}$ data in Table 1). We surmise that the salvaged cardiomyocytes would exhibit improved contractile function after stunning subsides, which could occur days to weeks after ischemic insult *in vivo* [40]. Further chronic myocardial I/R studies using Myr-PKCε- (5 μM) are needed *in vivo* to determine if stunning subsides after treatment. In toto, these results suggest that the 10 μM concentration was optimal

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to restore post-reperfused cardiac function and reduce infarct size.

We previously reported that 5 μM Myr-PKCε- resulted in 87% recovery of $+dP/dt_{max}$ initial baseline values using an I/R model in which isolated perfused rat hearts were subjected to 20 min of global ischemia followed by 5 min of reperfusion with a Krebs' solution containing 2 x 10^8 leukocytes and 40 min of leukocyte-free buffer [13]. By contrast, in the present study at the same PKCε- concentration (5 μM) no significant recovery of $+dP/dt_{max}$ and $-dP/dt_{min}$ compared to controls occurred. Infarct size was not measured in the previous study. We speculate that in a leukocyte augmented I/R model damage as evidenced by an impairment in function was synergistically produced by the leukocyte infusion and the 20 min ischemic period. Myr-PKCε- treatment possibly reduced leukocyte vascular adhesion and diapedesis into the myocardium, lowering leukocyte ROS release, and resulting in less cardiac dysfunction. Thus the almost complete recovery of post-reperfused cardiac function was due to blocking the leukocyte-mediated component of the damage. By contrast, the current study employed a longer 30 min ischemic period and no leukocytes. Nevertheless, in both I/R models, Myr-PKCε- given at reperfusion markedly improved post-reperfused cardiac function (i.e. $+dP/dt_{max}$ and $-dP/dt_{min}$). A putative mechanism for this effect in either I/R model is that treatment with Myr-PKCε- inhibits uncoupled eNOS activity, resulting in an increase in the bioavailability of NO and inhibition of degradation of NO produced from other sources such as hemoglobin and myoglobin nitrite reductase [13,34]. NO has been shown to promote a number of beneficial actions during normal cardiac activity and I/R injury, such as reduction in O_2 consumption, inhibition of Ca^{2+} influx, and improvement in the coupling efficiency between ATP synthesis and O_2 consumption [46-50]. In addition, constitutive NO stimulation of Na^+/K^+-ATPase has been reported to protect against mitochondrial membrane collapse and inhibits the formation of MPTP during reperfusion [51]. Collectively, these NO effects would improve cardiac function and reduce cell death (infarct size) during reperfusion. Interestingly, aliskiren is purported to increase NO bioavailability via increased eNOS phosphorylation when given prior to prolonged myocardial ischemia. This effect was blocked by N^G-nitro-L-arginine methyl ester [43]. It is possible that a threshold of NO bioavailability is obtained at the lower concentration (i.e. 5 μM) that is sufficient to limit infarct size independently from the effects of increasing concentrations of Myr-PKCε- that would improve post-reperfused cardiac function.

Several other animal studies have shown that chronic treatment with a cell permeable PKCε- was safe and effective in attenuating both cardiac and vascular remodeling following transplantation and angioplasty [45,52]. The purported mechanism mediating these chronic PKCε- effects was the inhibition of cytokine-induced metalloproteinase expression and subsequent associated extracellular fibrosis [45,53]. Consequently, acute administration of cell permeable PKCε- given at the onset of reperfusion may be a novel clinical therapy to attenuate cardiac contractile dysfunction and cell death following a heart attack, while chronic administration could be used to attenuate tissue remodeling following cardiac balloon angioplasty/stenting and transplantation.

Conclusion

Collectively, the results show that Myr-PKCε- treatment at the onset of reperfusion reduced infarct size at all concentrations tested while post-reperfused cardiac function markedly recovered at the higher concentrations. Thus, the data suggest that Myr-PKCε- treatment would be an effective clinical strategy to attenuate myocardial reperfusion injury in heart attack and transplant patients.

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