Pretreatment with a Novel Tri-Peptide Elicits Cardioprotective Effects in Myocardial Ischemia/Reperfusion Injury

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INTRODUCTION

Reperfusion of coronary blood flow to the ischemic heart following an acute myocardial infarction (MI), although necessary, may lead to myocardial ischemia/reperfusion (I/R) injury, resulting in cardiomyocyte death and compromised cardiac function [1]. The major cause of I/R injury is due to reactive oxidative species (ROS), which damage the mitochondria that comprise up to one-third of the heart volume and is one of the key producers of ROS [2] (Figure 1). The generated ROS leads to the loss of mitochondrial membrane potential and opening of the mitochondrial permeability transition pore (MPTP), leading to cardiac contractile dysfunction and increased infarct size [2].

There are currently no pharmacologic treatments that have been shown to clinically improve cardiac function and reduce infarct size in patients who have suffered from reperfusion-induced MI injury. Due to the role of mitochondria in I/R injury, treatment models targeting the mitochondria are a growing field in cardiovascular research.

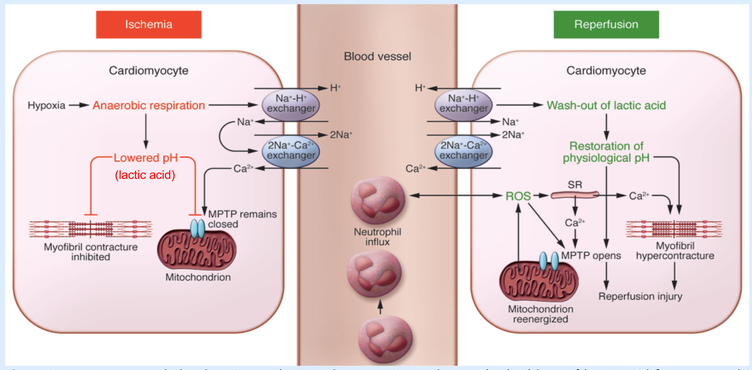


Figure 1. Acute myocardial ischemia results in a decrease in pH due to the build up of lactic acid from anaerobic conditions. The acidic conditions during ischemia prevent the opening of the mitochondrial permeability transition pore (MPTP) and cardiomyocyte hypercontracture at this time. Reperfusion results in washout of lactic acid, resulting in the rapid restoration of physiological pH, which releases the inhibitory effect on the MPTP opening, Ca^{2+} overload, and cardiomyocyte hypercontracture. The restoration of the mitochondrial membrane potential drives Ca^{2+} into the mitochondria, which can also induce MPTP opening and cardiac contractile dysfunction. Neutrophils accumulate in the infarcted myocardial tissue in response to the release of chemoattractants, and generate ROS. Adapted from Hausenloy and Yellon 2013 (1).

A novel tri-peptide (Phe-D-Arg-Phe-Amide, MW=468 g/mol) was found to attenuate ventilator induced diaphragmatic dysfunction (VIDD) when given as pretreatment. This tri-peptide is similar in structure to the SS-20 peptide (Phe-D-Arg-Phe-**Lys**-Amide), but does not contain the terminal Lys residue [3]. SS-20 has been shown to significantly reduce lipid peroxidation in the heart after prolonged ischemia suggesting inhibition of mitochondrial–derived ROS [4]. These small peptides also have a similar structure to some cardioprotective opioid receptor (delta (δ), kappa (κ), and mu (μ) agonists, which led us to study the role of opioid receptor activation as a putative mechanism for this tri-peptide's cardioprotective effects [5].

The heart predominantly expresses δ and κ opioid receptors throughout the atria and ventricles, however a recent study suggests the additional presence μ receptor [6-7]. Activation of opioid receptors is known to mediate cardioprotective effects during preconditioning [6-7]. Prior studies have proposed that the protective mechanism of pretreatment (i.e. preconditioning) involves the activation of mitochondrial K_{ATP} (mitoK_{ATP}) channels that attenuate MPTP formation and consequently protects the heart I/R injury [8].

Our experiments were designed to test whether pre- or post–treatment of tri-peptide is more efficacious in mitigating the deleterious effects of I/R injury and whether the opioid receptor pathway is involved with the cardioprotective mechanism using naloxone, a nonselective opioid receptor antagonist.

HYPOTHESIS

We hypothesize that this novel tri-peptide would significantly improve post-reperfused function and reduce infarct size in isolated perfused I/R rat hearts via activation opioid receptors, and therefore naloxone (broad-spectrum opioid receptor antagonist) will block the putative cardioprotective effect of tri-peptide.

METHODS

Male Sprague-Dawley rats (275-325 g, Charles River, Springfield MA) were anesthetized i.p. with sodium pentobarbital (60 mg/kg) and anticoagulated with heparin 1000 units. Hearts were isolated and studied using a modified Langendorff heart preparation as previously described [9]. All tri-peptide treated hearts received a dose of 50 μM prepared in plasma during the first five minutes of reperfusion via a syringe pump at 1 ml/min. Posttreatment trials received only tri-peptide prepared in plasma, pretreatment trials additionally received tri-peptide prepared in Krebs' buffer ± naloxone (Nlx, 10μM) during the last five minutes of baseline (pretreatment). Control hearts did not receive tri-peptide or naloxone. All hearts were frozen at -20°C for 30 min, sectioned into 2mm slices and incubated at 37° C in 1% 2,3,5-Triphenyltetrazolium chloride (TTC) to determine infarction size (Figure 2).

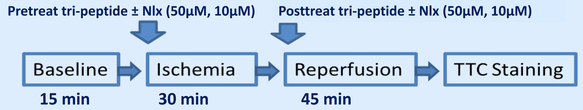


Figure 2. Flow diagram of the experimental protocol (above) and representative picture of isolated rat heart preparation (above right).

Statistical Analysis: All data in the figures are presented as means ± S.E.M. ANOVA analysis using Student-Neuman-Keuls test was used to assess statistical difference in cardiac function and infarct size between control I/R, pretreatment tri-pep + I/R, pretreatment tri-pep + Nlx + I/R, and I/R + posttreatment tri-pep hearts. Probability values of < 0.05 were considered statistically significant.

RESULTS

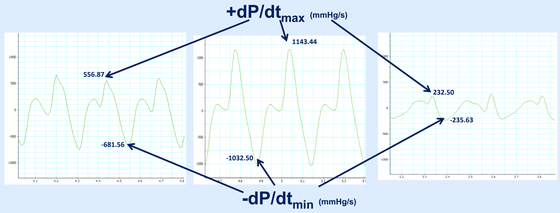


Figure 3: Representative tracings of the maximal rise of the left ventricular developed pressure (LVDP) [+dP/dT_{max}] and the maximal decline of LVDP [-dP/dT_{min}] for control I/R, Pretreat Tripep I/R, and Pretreat Tripep + Nlx I/R (left to right) hearts at 45 min reperfusion.

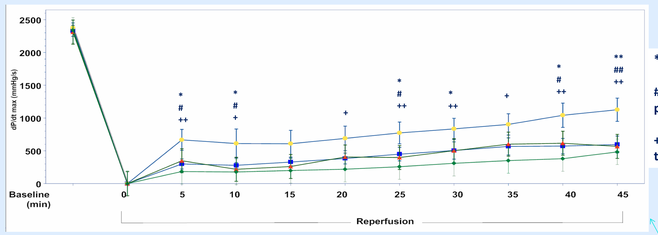


Figure 4. Time course of +dP/dt_{max} for Control I/R, Pretreat Tripep (50 μM) + I/R, PreTreat Tripep (50 μM) + Nlx (10 μM) + I/R, and I/R + Posttreat Tripep (50 μM) treated hearts. Pretreated tri-peptide hearts with naloxone only restored +dP/dt_{max} during reperfusion to 21 ± 3% compared to untreated controls, pretreated tri-peptide hearts, and I/R posttreated tri-peptide hearts which recovered to 25 ± 7%, 47 ± 8%, and 25 ± 7% of baseline values respectively at the end of the reperfusion period. There was no significant difference between the untreated controls and pretreated naloxone hearts.

*p<0.05 ;**p<0.01 vs. control I/R

#p<0.05; ##p<0.01 vs. I/R + posttreat tri-peptide

+p<0.05; ++p<0.01 vs. pretreat tri-peptide + Nlx+ I/R

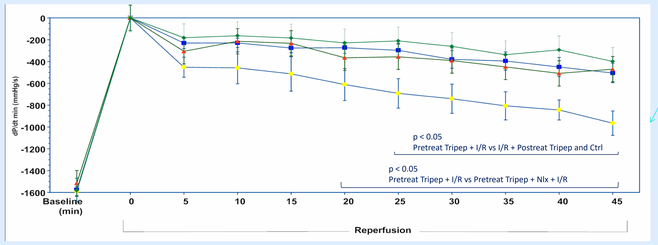
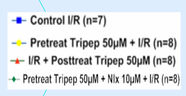


Figure 5. Time course of -dP/dt_{min} for Control I/R, Pretreat Tripep (50 μM) + I/R, Pretreat Tripep (50 μM) + Nlx (10 μM) + I/R, and I/R + Posttreat Tripep (50 μM) treated hearts. Pretreated tri-peptide hearts with naloxone only restored -dP/dt_{min} during reperfusion to 25 ± 4% compared to untreated controls, pretreated tri-peptide hearts, and I/R + posttreated tri-peptide hearts which recovered to 31 ± 7%, 61 ± 7%, and 31 ± 2% of baseline values respectively at the end of the reperfusion period.



RESULTS

Table 1. Cardiac function initial and final values for control I/R, Pretreatment + I/R, Pretreatment + Nlx (10 μM) + I/R, I/R + Posttreatment tri-peptide (50 μM) hearts. Pretreated hearts with tri-peptide and naloxone exhibited little improvement in post-reperfused cardiac function significantly at 45 min R in both LVDP and LVEDP measurements. LVDP recovered to 25 ± 4% compared to control I/R, pretreat + I/R, and I/R + posttreat tri-peptide hearts that recovered to 30 ± 7%, 55 ± 6%, and 31 ± 8% of initial values, respectively.

	Control I/R (n=7)	Pretreat + I/R (n=8)	Pretreat + Nlx + I/R (n=8)	I/R + Posttreat (n=8)
Initial LVESP (mmHg)	103.3 ± 3.1	100.9 ± 3.1	96.7 ± 3.5	100.8 ± 4
Initial LVEDP (mmHg)	7.9 ± 0.7	6.7 ± 0.8	5.2 ± 0.5	8.6 ± 1.6
Initial LVDP (mmHg)	95.4 ± 2.8	94.2 ± 2.7	91.5 ± 3.2	92.3 ± 3.4
Final LVESP (mmHg)	95.4 ± 3.5	105.6 ± 6.1	93.7 ± 5.5	94.2 ± 3.9
Final LVEDP (mmHg)	64.1 ± 4.6	*#++ 50.1 ± 7.4	70.2 ± 2.3	63.1 ± 5.1
Final LVDP (mmHg)	31.4 ± 6.7	**##++ 55.5 ± 6.0	23.5 ± 4.1	31.1 ± 8.5
Initial dP/dt_{max} (mmHg/s)	2367.6 ± 68.1	2376.7 ± 75.1	2340.7 ± 56.8	2311.1 ± 94.9
Final dP/dt_{max} (mmHg/s)	596.6 ± 138.6	**##++ 1126.8 ± 177.2	483.4 ± 79.6	567.9 ± 171.2
Initial dP/dt_{min} (mmHg/s)	-1612.5 ± 85.6	-1590.5 ± 45.7	-1581.1 ± 88.2	-1515.2 ± 87.0
Final dP/dt_{min} (mmHg/s)	-505.6 ± 78.3	**##++ -965.5 ± 112.1	-399.2 ± 58.5	-470.2 ± 96.1
Initial Coronary Flow (mL/min)	19.4 ± 2.2	17.7 ± 0.9	18.7 ± 1.9	20.3 ± 1.9
Final Coronary Flow (mL/min)	7.8 ± 1.0	9.6 ± 1.5	6.7 ± 0.4	8.0 ± 0.9
Initial Heart Rate (BPM)	266.9 ± 9.5	287.8 ± 8.1	283.7 ± 5.8	271.5 ± 7.9
Final Heart Rate (BPM)	259.2 ± 11.2	276.3 ± 12.6	257.1 ± 8.5	273.9 ± 22.5

*p<0.05 vs. control I/R. **p<0.01 vs. control I/R.
#p<0.05 vs. I/R + posttreat. ##p<0.01 vs. I/R + posttreat.
+p<0.05 vs. pretreat + Nlx + I/R. ++p<0.01 vs. pretreat + Nlx + I/R.

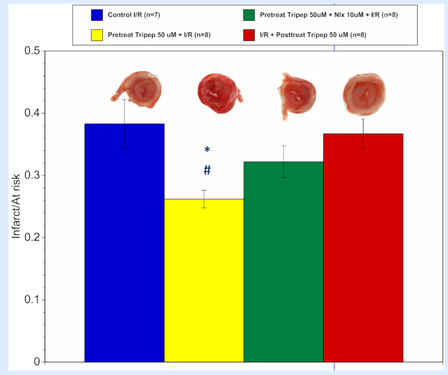


Figure 6. Weight ratios of infarcted heart tissue vs at risk left ventricular heart tissue in I/R as determined by TTC staining. Pretreat Tripep + Nlx + I/R (32.1 ± 0.03) showed decrease in infarct size, but with no significant difference between control I/R (38.3 ± 0.04).

CONCLUSION

Pretreatment with tri-peptide significantly restored both post-reperfused cardiac function and reduced infarct size compared to untreated control I/R hearts, I/R + posttreated tri-peptide hearts, and pretreated tri-peptide + naloxone + I/R hearts. The improvement in post-reperfused heart function is mostly due to a decrease in LVEDP.

Naloxone blocked the cardioprotective effects of tri-peptide during pretreatment, suggesting that the mechanism of tri-peptide likely involves preconditioning via opioid receptor activation which in part involves opening of mitoK_{ATP} channels.

The results from this study suggest that novel tri-peptide would be an effective treatment that can be given to coronary bypass or organ transplant patients to restore heart function and reduce reperfusion injury in heart tissue.

Future studies will aim to identify the opioid receptor subtype mediating the preconditioning effect of tri-peptide. Additional studies will delineate whether the pretreatment mechanism of opioid receptor activation involves the opening of mitoK_{ATP} channel by blocking the channel with 5-hydroxydeconoate (5-HD).

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