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Preconditioning of Healthy, Stunned, Infarcted, Hypertrophied and Failing Hearts: Role of Conditioning Reserve in Supplemental Cardioprotection

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Abstract

Background: Classical Ischemic Preconditioning (IPC) limits Myocardial Infarction (MI) in previously normal subjects and naïve hearts but not in diseased hearts. The current studies have investigated myocardial preconditioning in five clinically relevant models of stunned, infarcted, Spontaneous Hypertrophied (SH) and End-Stage Failing (ESF) hearts.

Methods: Global Myocardial Stunning (GMS) was created in dogs, on-pump. Heart performance (Stroke-Work/End diastolic length) was used as a primary end-point. Eight dogs were subjected to six interrupted episodes of Warm Global Aortic Cross-Clamping (WACC) and reperfusion (10 x 10 min) compared to sustained WACC and reperfusion (60 x 60 min). SH dog hearts (n=11) were subjected to GMS, on-pump with or without infusion of adenosine, an IPC memetic. Rabbit model (n=75) of acute Myocardial Infarction (MI) was used to determine the effects of prior acute MI on reinfarction of the same, or remote, vascular bed and whether IPC provides supplementary cardioprotection. Canine model of heart failure (n=33) and UM-X 7.1 strain hamsters (n=28), develops idiopathic dilated cardiomyopathy with age, were used to determine whether IPC limits MI in failing hearts compared to non-failing hearts.

Results: In dogs, the first GMS caused ATP depletion (32%) and dysfunction (42%). Five episodes of GMS did not cause cumulative contractile dysfunction (stunning), confirming stunning-induced contractile preconditioning ($p<0.05$ vs. sustained 60 min GMS). In rabbit model of acute MI, re-infarction of the same vascular bed did not expand the infarct size. Prior acute MI significantly reduced the infarct size in a remote area ($p<0.05$). Dilated cardiomyopathy canine and hamster hearts had significantly smaller infarct size in the absence of prior IPC. KATP abolished infract size limitation in MI-induced preconditioned hearts.

Conclusion: Stunned, infarcted, hypertrophied and failing hearts are perpetually preconditioned and utilizing maximal preconditioning reserve against myocardial stunning or MI. IPC, per se, provides no or limited supplementary cardioprotection in diseased hearts, thus offering the basis of biological explanation for the adaptive response "MEMORY" to ischemic stress and disease.

Keywords: Ischemic preconditioning; Myocardial stunning; Infarction; Re-infarction; Infarct size; Remote preconditioning; Preconditioning reserve; Disease; Cardiac function; Stroke work/end diastolic lengthens; ATP; Adenosine

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Introduction

Early empirical observations suggested that close events of angina pectoris prolong patients' survival, however, the mechanisms for this response remains unknown. In the 1970's, repetitive episodes of coronary artery occlusions and reperfusion significantly reduced the rate of ATP depletion and limited infarct size expansion in previously normal canine models with naïve canine hearts [1-5]. In the 1980's, these observations have been reproduced. Numerous normal small animal models were used to elucidate biochemical, signaling and molecular mechanisms of ischemic preconditioning using the infarct size as a primary end-point [6-12]. Leading principle investigators rushed to postulate that ischemic preconditioning, in normal animal and hearts, was the most powerful modality for cardioprotection against myocardial infarction, as the primary end-point [13]. The same leading investigators failed to demonstrate that association between the

reductions of infarct size and improved cardiac performance. It was not clear whether ischemic preconditioning is a model dependent, or it is really a generalizable biological phenomenon [14-16].

There are no studies found on ischemic preconditioning in diseased animal models with ischemic or infarcted hearts. On the other hand, augmentation of myocardial conditioning in diseased hearts and patients have not received an equal attention and later abandoned due to lack of supporting experimental and clinical efficacy of ischemic preconditioning in patients with metabolic or ischemic diseases undergoing cardiac surgery. It was logic to first document whether ischemic preconditioning is clinically efficacious in cases of ischemic syndromes and cardiomyopathy prior to heavily investigating signaling and molecular mechanisms of necrosis and death. Research efforts should have been directed to the most likely needed supplemental cardioprotection in cases of myocardial stunning, hibernation, ST-Segment Elevation Myocardial Infarction (STEMI), ventricular hypertrophy and dilated failing hearts undergoing invasive transcatheter endovascular interventions or surgical revascularization. In 1993, we received an RO1 NIH grant to investigate cardioprotection of the failing hearts undergoing cardiac surgery. Early preliminary studies demonstrated that, in the absence of ischemic preconditioning, failing hearts exhibited significantly smaller infarct size and are much tolerant to myocardial infarction and cannot be supplementary preconditioned against infarction with ischemic preconditioning stimuli [17-20]. Other studies have documented failure of classical ischemic preconditioning under clinical settings in patients with different types of diseases [13,21-28] with no further efforts have made to elucidate why diseased heart could not be ischemically preconditioned.

The current studies were undertaken to determine, once and for all, the feasibility of augmenting myocardial tolerance, if any, to ischemia in four clinically relevant animal models: 1) an acute global myocardial stunning (dogs); 2) spontaneously hypertrophied (dogs); 3) a regional myocardial infarction in rabbit model of acute MI; and 4) canine hearts and in models of congestive failure in and in an idiopathic dilated cardiomyopathy UM-X 7.1 hamsters. Results reproducibly demonstrated that diseased hearts are perpetually preconditioned, depending on residual conditioning reserve, and probably exhausted their preconditioning reserve with no or little benefit with supplementary brief ischemic stimuli. Selective blockade of KATP channel abolished myocardial tolerance to an acute MI challenge in both healthy and in diseased hearts suggesting involvement of KATP channels in the adaptive conditioning response, not only in health, but also in disease. Our results provide biological explanation for the adaptive responses "Memory" against ischemic stress and in health and disease.

Materials and Methods

Biochemical reagents and inhibitors were purchased from Sigma-Aldrich Chemical Company (St. Louis, Mo), or otherwise stated.

Animal models and experimental designs

The following studies conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health and approved by the IACUC Committee in VCU. UN-X 7.1 strain hamsters were purchased from Dr. Gaétan Jasmin, Department of Pathology, Faculty of Medicine, University of Montreal, and Montreal, QC, Canada.

Experimental design and protocols

The current work consists of four arms of separate experimental studies to determine the efficacy of ischemic preconditioning in four clinically relevant animal models of myocardial stunned in normal and spontaneous hypertrophied, infracted, and failing hearts compared to normal control hearts.

Canine model of global myocardial stunning, on-pump

Sixteen microfilaria-free adult mongrel dogs, of either sex weighing 17 kg to 25 kg, were used in this study. Dogs were anesthetized initially with 35 mg/kg sodium pentobarbital intravenously and maintained with 10-mg/kg boluses of sodium pentobarbital. Dogs were intubated and ventilated with 95% oxygen and 5% carbon dioxide. Surgical procedures and instrumentations were performed as described previously [29,30]. Briefly, after median sternotomy, the right phrenic nerve was transected, and the sinoatrial node was crushed, and the heart was paced at rate of 150 bpm in order maintain equal cardiac work. Body and heart temperatures were maintained at 37 ± 1°C. Dogs were heparinized with an intravenous injection with porcine-based heparin (400 U/kg) as an initial bolus followed by 200 U/kg/hr (Elkins-Sinn Inc, Cherry Hill, NJ), then placed on cardiopulmonary bypass using a membrane oxygenator (Medtronic, Minneapolis, MN). The extracorporeal circulation was primed with non-cross-matched homologous blood from a bleeder dogs (n=16), and an aortic cannula and a two-stage venous cannula were introduced into the right atrium and the inferior vena cava, respectively. The azygous vein was occluded, and the left ventricle was vented at the apex. The mean arterial reperfusion pressure was maintained at 60 to 65 mmHg while on bypass. Arterial blood gases, pH, and hematocrit were determined routinely and maintained at the following levels: pressure of oxygen, 100 to 140 mmHg; pressure of carbon dioxide, 30 to 40 mmHg; pH, 7.32 to 7.48; and hematocrit, about 30%.

Assessment of left ventricular performance

Left ventricular performance was assessed, off bypass pump, from the slope of the relationship between Stroke-Work and End Diastolic Length (SW/EDL), as a sensitive and load-independent index of contractility, using pulse transit sonomicrometry (Triton Technology, San Diego, Calif) and Millar balloon-tipped pressure transducers as previously described [29,30]. One pair of LTZ-piezoelectric hemispheric crystals was sutured to the anterior and posterior of the epicardial surface of the left ventricle wall in the minor axis (40 mm to 60 mm). Analog data were digitized at 200 Hz and stored on a personal computer hard disk and analyzed by interactive Crunch software developed in Andrew S. Wechsler's laboratory at Duke University Medical Center. Data acquisition was performed at varying preloads, by gradually emptying of the left ventricle via venous drainage, thus creating diminishing cardiac work loops within 20 to 40 cardiac cycles.

Myocardial stunning in a normal canine model, on-pump

Part of this study on normal animals was previously published [31] and presented here for additional support for cardiac function preconditioning with prior episode of myocardial stunning. An acute global myocardial stunning was created at (37°C) by 10 minutes of aortic cross clamping (WACC) and 10 minutes of reperfusion, while on cardiopulmonary bypass (n=8). After the first WACC, five consecutive episodes of 10 minutes of global ischemia and 10 minutes reperfusion each were applied. After six episodes of intermittent stunning, hearts were reperfused for 120 minutes. For comparison, dogs (n=8) were subjected to 60 minutes of sustained WACC, on-

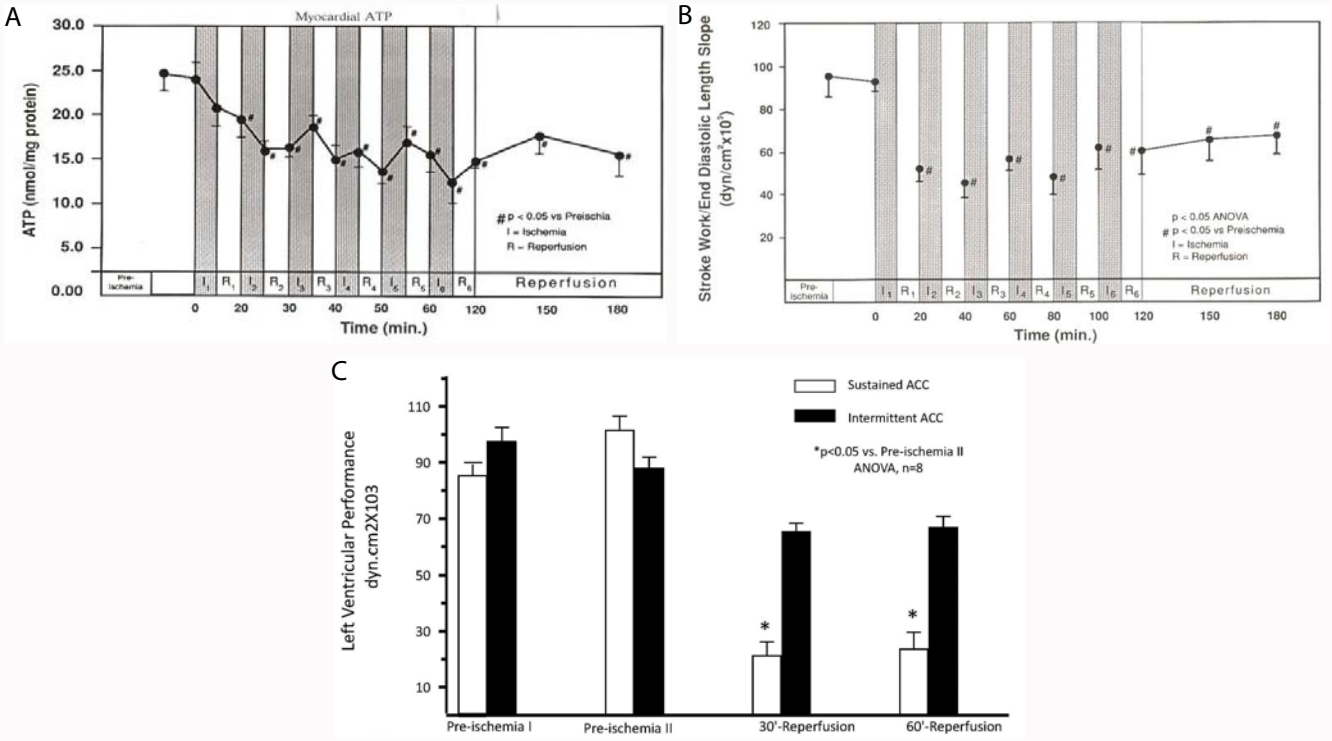

Figure 1: Myocardial Stunning-Induced Preconditioning in Dogs, on-Pump.

Figure 1A: Myocardial ATP during Repeated Stunning.

The effect of intermittent ischemia on ATP with and without nucleoside trapping. Effect of intermittent global normothermic ischemia and reperfusion on myocardial ATP is depicted. There were significant reduction in myocardial ATP after the first episode of stunning but did not change during five additional episode of myocardial stunning. *p<0.05 *vs.* baseline value.

Figure 1B: Heart Performance and Repeated Stunning.

The effect of intermittent global normothermic ischemia and reperfusion on left ventricular function is depicted. There was a significant difference between cardiac function after one episode of myocardial stunning. It is interesting to note that cardia performance recovery was not affected during five consecutive episodes of stunning. *p<0.05, ANOVA, n=8.

Figure 1C: Heart Performance with or without Repeated Stunning.

The effect of intermittent global normothermic ischemia and reperfusion on left ventricular function compared to sustained 60 minutes of warm global aortic cross clamping is depicted. There was a significant difference between cardiac function after six episode of myocardial stunning and 60 minutes of sustained ischemia. *p<0.05, ANOVA, n=8.

pump and 120 minutes of reperfusion. Transmural myocardial biopsy specimens were obtained for adenine nucleotides pathway metabolism and analyzed by HPLC [29-32]. Cardiac function was measured at the end of each period of 10 minutes of reperfusion, during interrupted ischemia and reperfusion and during sustained 120 minutes of reperfusion.

Myocardial stunning in a canine model of spontaneous idiopathic hypertrophied cardiomyopathy

A colony of dogs (n=11) with chronic spontaneous idiopathic hypertrophied cardiomyopathy were obtained as a gift from an organized dog race track, Ohio, were used in this study. All dog had global cardiac hypertrophy, with left ventricular wall thickness was >33.4 mm. Dogs were anesthetized, intubated ventilated and place of cardiopulmonary bypass, using blood from normal donor dogs (n=11) and instrumented to measure heat performance using sonomicrometry as describe above. After collecting baseline functional data and obtained transmural myocardial biopsy, dogs were divided into two groups with (n=6) or without (n=5) three boluses of intracoronary infusion of adenosine (100 μM) without adenosine deaminase. The vehicle or adenosine was administered 5 min prior to applying the aortic cross clamping. All hearts were subjected to 30 min warm global myocardial ischemia, on-pump, and 120 minutes reperfusion. Since adenosine was found to be an ischemic preconditioning memetic in normal hearts, we determined whether adenosine would protect against myocardial stunning induced by aortic cross clamping and reperfusion in spontaneous hypertrophied canine hearts.

Rabbit model of myocardial infarction, re-infarction and remote infarction

Seventy-five normal adult New Zealand male rabbits (3 Kg to 4 Kg) were used in this study. Were used in this experimental protocol. Each rabbit was anesthetized with pentobarbital (40 mg/Kg, i.v.) and maintained at 10 mg/Kg as needed, intubated, ventilated, with room air supplemented with oxygen 95% and carbon dioxide 25%, and a median sternotomy was performed and divided into eleven groups (n=5 each) according to the first coronary artery occlusion. The first acute Myocardial Infarction (1st MI) was created in three groups of normal rabbits by occluding the right (RCA, n=5), the Circumflex (CFX, n=5), or the first diagonal of the Left Anterior Descending coronary artery (LAD, n=5) for 30 minutes and reperfused for 120 minutes. The infarct size normalized per area at risk in the corresponding ventricle. To determine whether a second sublethal ischemia (MI), created by re-occluding the same coronary artery (2nd MI) separated by 10 minutes reperfusion, could expand the pre-

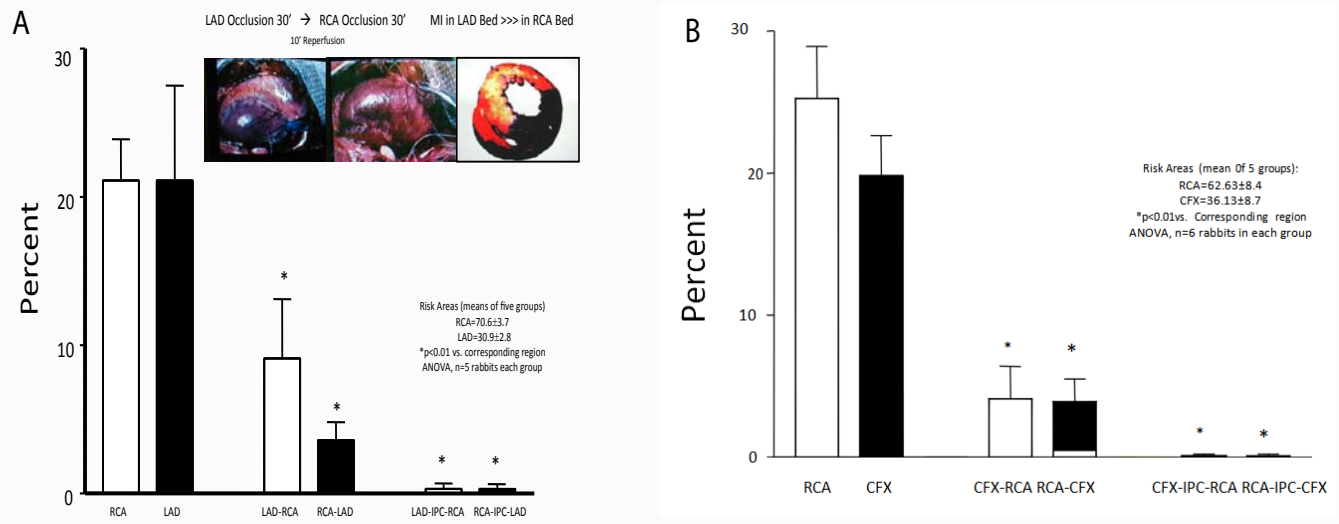

Figure 2: Myocardial Infarction Preconditions Rabbit Hearts against Secondary MI.
Figure 2B: Figures (2A-2B) show the effect of an acute myocardial infarction in one vascular bed on a secondary MI in a remote vascular bed and the effects of ischemic preconditioning, created by two episodes of coronary artery occlusion and reperfusion, on infarct size of the secondary MI. *p<0.05, ANOVA, n=8.

existing infarct size in the same vascular or a remote. To determine whether occluding a virgin remote vascular bed after the 1st MI provides MI-induce cardioprotection. In CFX-RCA group (n=5), the CFX coronary artery was occluded first for 30 minutes and reperfused for 10 minutes (1st MI) before applying a second remote MI by occluding the RCA for 30 minutes and reperfusion for 120 minutes (2nd MI). In RCA-CFX group (n=5), the RCA was occluded first 30 minutes and reperfused for 10 minutes before initiating the CFX MI (2nd MI). In LAD-RCA group (n=5), the LAD was occluded first (1st MI) before RCA 2nd MI and vice versa RCA-LAD groups (n=10). To determine whether, two short ischemic preconditioning of a virgin remote coronary artery after the 1st MI further cardioprotect against 2nd MI, a group was created to induce the 1st MI by occluding the LAD for 30 minutes and then reperfused for only 10 minutes before initiating classical Ischemic Preconditioning (IPC) by occluding the RCA (2X5' LAD + 10' R) followed by the RCA 2nd MI, i.e., 30 minutes RCA occlusion and 120 minutes reperfusion (n=5). To determine the area at risk of the 2nd MI, Evans blue (1%) was administered by direct injection into the ascending aorta after applying the aortic cross clamp and snaring the coronary artery of interest for the 2nd MI. At the end of each experiment, the heart was dissected, cooled and sliced into thin sections and incubated in 1% Trichlor Tetrazolium Chloride (TTC) solution at 37°C to stain the viable myocardium areas. Infarct size was determined by the ratio of infarcted area divided by the corresponding risk area. To determine whether mitochondrial ATP-sensitive potassium channel Mito-K-ATP is involved in MI-induced preconditions against second acute remote MI, The vehicle solution or Mito-K-ATP blocker, 5-hydroxy-decanoate (5 HD, 5 mg/Kg, iv, n=5) was infused prior to the first MI. Hemodynamic parameters and the heart rates were monitored to the completion of study. At the end of each experiment, the area at risk/LV area and percent of infract size/are at risk were measured. Exclusions involve low mean arterial pressure and hemodynamic stability was compromised.

Canine model of end-stage congestive heart failure

Under aseptic conditions, anesthetized healthy dogs were anesthetized instrumented with a transvenous pacemaker lead was placed in the right ventricle. The pacing lead was positioned in the right ventricular apex under fluoroscopic guidance. Prophylactic antibiotics were administered before skin incision and one dose postoperatively. After recovery period (2 days), the pacemaker was activated to 250 bpm/min and the animal was paced for 8 weeks, inducing mild to severe cardiomyopathy, respectively. Dilated cardiomyopathy was documented with echocardiography and electro-cardiograms. Heart failure was created by chronic rapid pacing (240-250 bpm, 6-8 weeks). Progression of ventricular remodeling and congestive heart failure were confirmed by echocardiography studies. Severe congestive heart failure manifested by ascites, lethargy, anorexia, and pulmonary edema is evident within 6 to 8 weeks of pacing. The overall average values of the ejection fraction ranged between 8 and 25%.

From our early previous experience with canine model of congestive heart failure, it was difficult to use an intravenously injection of pentobarbital for anesthesia to or placed on cardiopulmonary bypass circulation (n=2). Therefore, animals were anesthetized and maintained with Isoflurane (1% to 2%, using a vaporizer) were intubated, ventilated with room air supplemented with oxygen 95% and carbon dioxide 25% and median sternotomy was performed. Age-matched control dogs (n=8) were prepared for surgery.

Thirty-three dogs survived chronic rapid pacing and additional four dogs expired early during rapid pacing. The mortality rate was about 15% (4 dogs) during 8 weeks of rapid pacing. Anesthetized normal (n=18) dogs and failing dogs (n=33) were subjected to MI challenge including an index ischemia off-pump by occluding the LAD coronary artery followed by 4 hrs reperfusion. Because all animal was at end-stage congestive heart failure, we decided to subject animals to shorter period of index ischemia a shorter period 45 minutes of index ischemia was implemented off-pump. The infarct size was found to be very small (<2%) in failing hearts compared to age-matched previously healthy dogs, despite that fact that the risk areas were adequate (30% to 40% of the LV areas). The period of index ischemia was increased to 60 and then to 90 minutes of LAD occlusion and 4 hours reperfusion. Age-matched animals were used as control, in which the infarct size was consistently reproduced by about 30% of the risk area of the left ventricle. Colored microspheres were used to measure collateral blood at baseline and during LAD occlusion in all animals [33]. ATP pool and HSP70 were determined

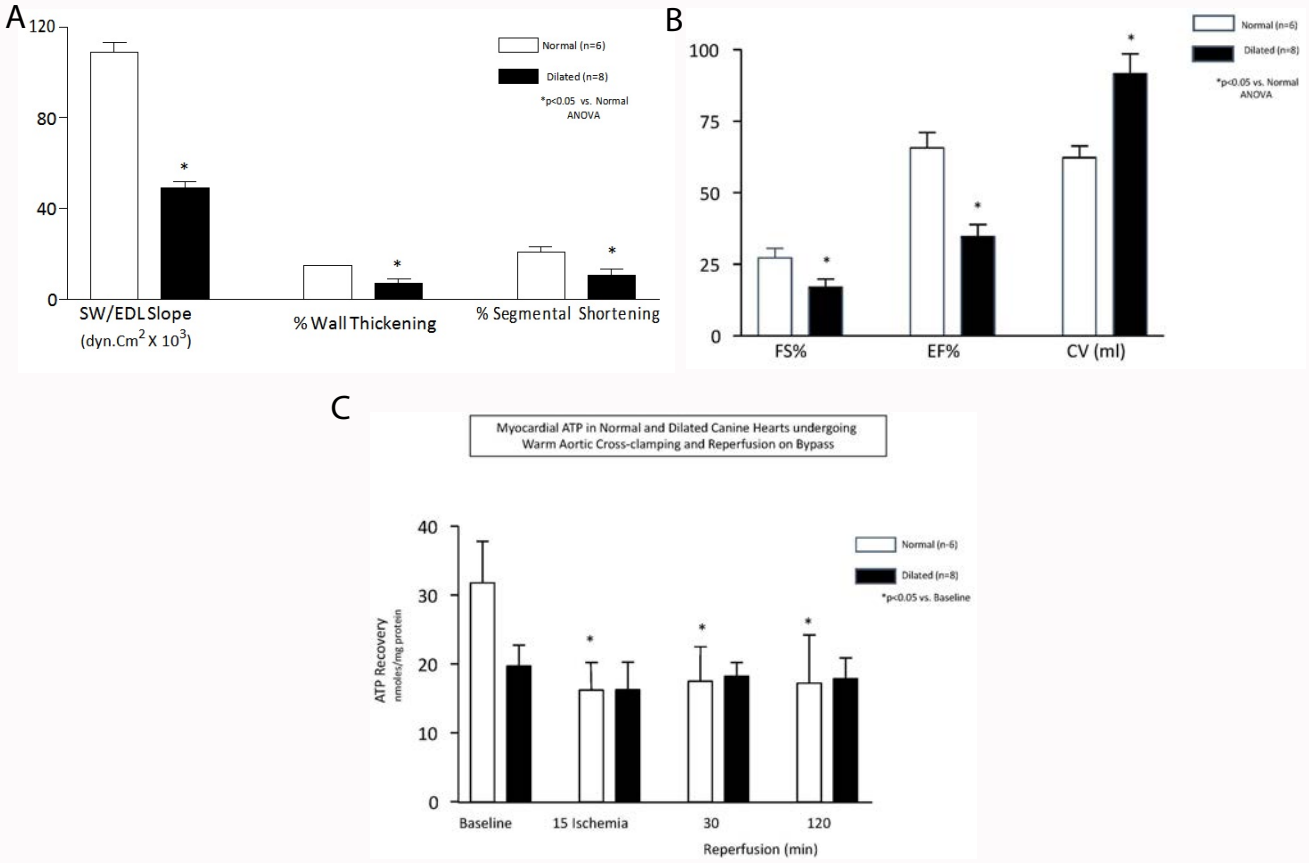

Figure 3: Canine Model of Congestive Heart Failure.
Figure 3A-3B: Documents the differences in heart performance (SW/EDL), wall thickening (WT%), fractional shortening (FS%), ejection fraction (EF%), and end-diastolic volume (chamber volume CV ml). **Figure 3C:** Difference of myocardial ATP in normal and dilated hearts in shown in Figure (3C) in normal and dilated canine hearts. *p<0.05.

in tissue biopsies. Differential staining with Monastral blue and TTC staining has delineated risk and infarct size. The effects of ischemic preconditioning were measured in limited number of animals (n=5) by 5 minutes LAD occlusion and 10 minutes reperfusion prior to challenge with a sustained LAD occlusion 90 minutes ischemia and 4 hrs reperfusion. The role of mitochondrial-KATP channel in the infarct size was determined in healthy (n=5) as well as failing dogs (n=5) was determined by intravenous infusion of 5-hydroxydecanoate (5-HD) 5 mg/Kg in a vehicle solution of saline.

Hamster model of idiopathic dilated cardiomyopathy (IDCM)

The Syrian Bio UM-X 7.1 hamster strain, a mutant that develops an idiopathic dilated cardiomyopathy with age (within 200 days) [34] were used as another model of idiopathic dilated cardiomyopathy in the absence of chronic rapid pacing the Bio UM-X 7.1 hamster strain was purchased from Dr. Gaétan Jasmin, Department of Pathology, Faculty of Medicine, University of Montreal, Montreal, QC, Canada. Age-matched normal wild (n=15) and Bio UM-X 7.1 hamster (n=28) were anesthetized with pentobarbital (40 mg/kg, i.v.), ventilated *via* intratracheal tube and a median sternotomy was performed. An acute MI was created in hamsters by occluding the first diagonal of the left ascending anterior (LAD) coronary artery for 30, 45 or 60 minutes and reperfused for 2 hrs. Ischemic preconditioning protocol involved 5 minutes coronary artery occlusion and 10 minutes reperfusion prior to MI challenge.

Experimental protocol started with 30 minutes coronary artery occlusion and 2 hrs reperfusion. In preliminary experiments using idiopathic dilated hamster, the infarct size was not detectable after 30 minutes of LAD occlusion followed by 2 hrs reperfusion. Prolonging the duration of acute coronary artery occlusion to 45 or 60 minutes resulted in measurable infarct sizes. Therefore, the three durations of ischemia were selected for normal and dilated hamsters. To determine whether adenosine receptors or sarcolemma ATP-sensitive potassium channels (KATP) are involved in ischemic preconditioning in UM-X 7.1 hamster hearts, intraperitoneal injection of 7.5 mg/Kg of 8-(p-sulfophenyl) theophylline) (8-SPT), a non-selective adenosine receptor blocker or Glibenclamide (Glyburide), 5-chloro-N-[2-[4-(cyclohexylcarbamoylsulfanoyl)phenyl]ethyl]-2-methoxybenzamide (0.3 mg/Kg, iv) were administered 5 minutes before sustained ischemia. Glibenclamide is an antidiabetic sulfonylureas medicine known as glyburide.

Statistical Analysis

Data are presented as mean ± standard error of the mean. For myocardial stunning study, sequential measurements were compared using repeated-measures analysis of variance and Turkey post hoc tests using SAS (version 6.02; Statistical Analysis System Institute, Cary, NC). ANOVA was used to determine statistical significance using Prism software in the other studies. Differences were considered significant if the probability value for comparison of least squares means was less than 0.05.

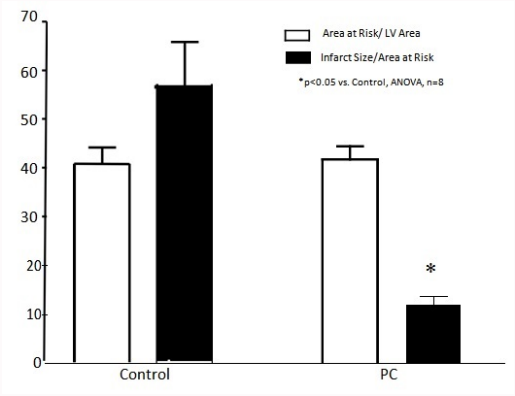

Figure 4A

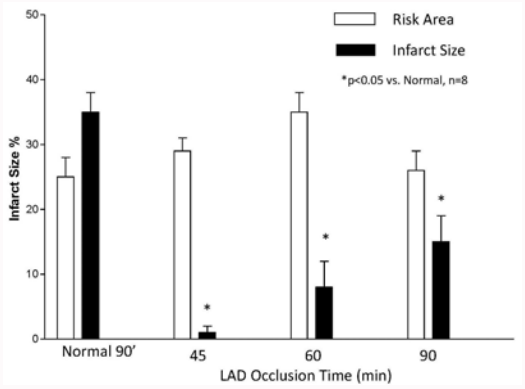

Figure 4B

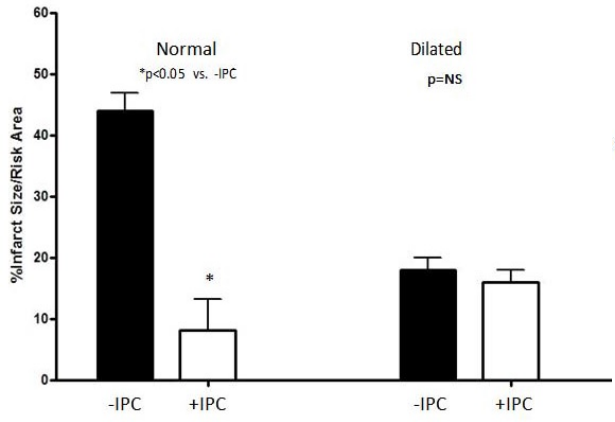

Figure 4C

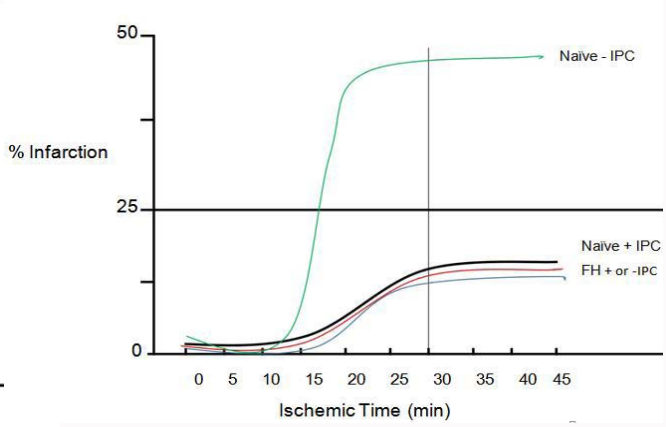

Figure 4D

Figure 4E

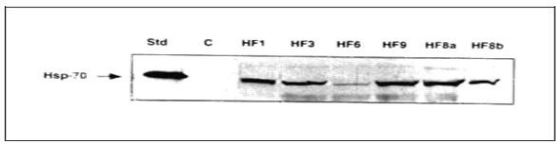

Figure 4: Myocardial Conditioning in a Canine Model of Congestive Heart Failure.
Figure 4A shows the effect of ischemic preconditioning in normal canine hearts. *p<0.05 *vs.* non-preconditioned hearts.
Figures 4B-4D demonstrate that failing hearts at the end-stage of dilated cardiomyopathy are more tolerant to a sublethal episode of an acute myocardial infarction challenge compared to naïve hearts in normal dogs. *p<0.05 *vs.* normal hearts.
Figure 4B document smaller infarct size in dilated hearts at 45, 60 and 90 minutes of LAD occlusion and reperfusion. Figures (4C,4D) summarize the effect of ischemic preconditioning in normal and failing diseased canine hearts. *p<0.05 *vs.* normal group, n=6-8).
Figure 4E: Expression of Heat shock Protein 70 (HSP 70) in Normal and Dilated Canine Hearts. Figure 4D illustrates results from Western Blot of myocardial tissue obtained from chronically paced hearts, before or after acute myocardial ischemia. All chronically paced hearts expressed the inducible HSP 70 determined by the inducible HSP 70 antibodies (810 Stress Gen Biotechnologies Corporation, Victoria, BC, Canada) with minimal expression of the constitutive HSP 70 (<1 ± 1) (using antibodies 815, Stress Gen Biotechnology). STD=pure HSP 70, C=Control =normal heart was not subjected to chronic rapid pacing, HF= dogs with signs of heart failure induced by chronic rapid pacing (8 weeks).

Exclusions

Hemodynamic parameters were continuously monitored. Exclusions involved animals with significantly low hemodynamic parameters or premature death during induction of anesthesia or more than 50% reduction in systolic pressure and heart rate. No medications were used to support heart rates or pressure. Dogs with congestive heart failure were sensitive to a single intravenous injection of sodium pentobarbital resulting in death (n=2). Mortality was less than 6% in healthy rabbits and in dogs and hamster with heart failure. No mortality occurred in myocardial stunning studies in healthy and hypertrophied and failing dogs.

Results

The anti-infraction induced by Ischemic Preconditioning (IPC) has been reproducible in naïve un-stressed animals and hearts; however its clinical efficacy remains disappointed and sometimes misleading. Therefore, we determined the effects of ischemic preconditioning in clinically relevant models: 1) myocardial stunning in normal; 2) spontaneous hypertrophied hearts, on-pump, 3) acute myocardial re-infarction in rabbit model. 4) Myocardial infarction in canine and hamster models of chronic heart failure.

Myocardial sunning-induces functional preconditioning in normal canine model, on-pump

Classical cardiac ischemic preconditioning increases tolerance

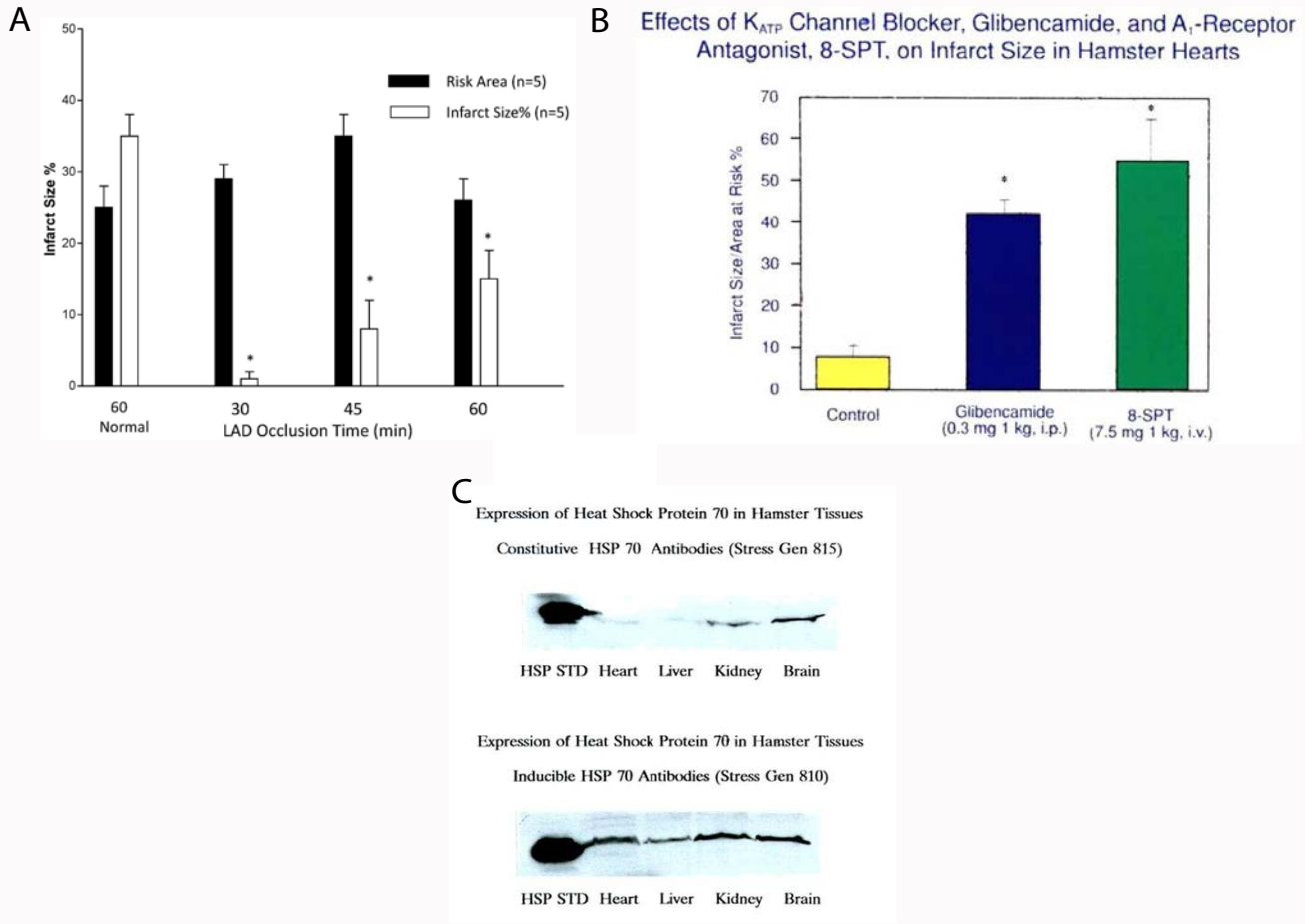

Figure 5: Myocardial Conditioning in Dilated UM-X 7.1 Hamster Hearts.
Figure 5A: Demonstrate that dilated UM-X 7.1 hamster hearts had smaller infarct size after challenged with different periods (30, 45 or 60 minutes) of sustained LAD occlusion and 120 minutes reperfusion.
Figure 5B: Depicts the effects of KATP channel and adenosine receptors blockers in infarct Size in dilated UM-X 7.1 hamster hearts subjected to 60 minutes of sustained ischemia and 120 minutes of reperfusion. *p<0.05 *vs.* baseline infarct size in the absence of blocker.
Figure 5C: Expression of Constitutive and Inducible Heat shock Protein 70 (HSP 70) in UM-X 7.1 Dilated Hamster Hearts, liver, kidney and brain.
Figure (5C) illustrates expression of the constitutive and induceble HSP 70 in dilated UM-X 7.1 Hearts, liver and kidney from Western Blot of myocardial tissue. HSP 70 antibodies (810 Stress Gen Biotechnologies Corporation, Victoria, BC, Canada) with some expression of the constitutive. STD= pure HSP 70, C=Control =normal heart was not subjected to chronic rapid pacing, Dilated UM-X 7.1 hamster hearts.

of normal hearts to myocardial infarction induced by an acute sublethal ischemic challenge. The infarct size has been considered as a gold standards end-point for classical ischemic preconditioning particularly in rabbits and murine models. Based on the assumption that small infract size must be accompanied with recoverable cardiac performance, the correlation between infarct size and contractile recovery was not proportional to infarcts size limitation. The role of myocardial stunning, associated with ischemic preconditioning trigger, in ischemic preconditioning was prematurely ruled out on the basis that administration of dobutamine augments hemodynamic and myocardial contractility in normal as well as ischemically preconditioned hearts regardless of the observed infarct size [35,36].

To pursue these issues, we determined whether an episode of myocardial stunning, induced by 10 minutes global myocardial ischemia and 10 minutes reperfusion, can precondition against additional five consecutive episodes of 10 minutes ischemia and 10 minutes reperfusion in a canine model of global warm aortic cross clamping on-pump. As an end-point, heart performance was measured at the end of every 10 minutes reperfusion after each episode of ischemia. Results clearly demonstrated that the first 10 minutes of warm aortic cross clamping followed by 10 minutes reperfusion, caused global myocardial stunning as evident by loss of ATP (30% to 40%) (Figure 1a) and of cardiac function (42%) (Figure 1b) compared to baseline of normal function ($p<0.05$). Five additional episodes, of 10 minutes ischemia and 10 minutes reperfusion, did not result in a cumulative cardiac dysfunction (stunning). After the first episode myocardial stunning, cardiac function and myocardial ATP remained about 60% to 70% of baseline levels, despite of repeated stunning (Figure 1a, 1b). Since cardiac function and ATP were depressed after the first episode of stunning, it was logic to relate the subsequent recovery of function and ATP to the values after the first stunning and rather not of the basis of the baseline of normal values. Cardiac function recovery was normalized per baseline function as well as per cardiac function after the first episode of 10 minutes aortic cross clamping and 10 minutes reperfusion. Accordingly, it was intriguing to note that the recovery of cardiac function and ATP values were actually 100%, suggesting that a period of ischemia that causes stunning also preconditions against repeated ischemic events. On the other hand, subjecting dogs with normal hearts to 60 minutes of a sustained period of WACC and 60 minutes reperfusion resulted

Table 1: Left Ventricular Performance (SW/EDL Slope, dyn/cm2X103).

	Baseline	30'Isch	30'Rep	120'Rep
Control	82.7 ± 5.4	-	43.3 ± 8.9	48.6 ± 8.2
Adenosine	77.0 ± 11.0	-	44.6 ± 13.6	16.8 ± 2.0 * #
		Myocardial ATP (nmol/mg protein)		
Control	35.6 ± 1.5	16.7 ± 1.9*	16.5 ± 2.1*	15.8 ± 2.3*
Adenosine	33.6 ± 3.8	15.4 ± 2.7*	17.0 ± 2.4*	18.3 ± 3.3*

*p<0.05 *vs.* baseline, #p<0.05 *vs.* Control group, mean ± SEM, ANOVA. Results demonstrated failure of adenosine, an ischemic preconditioning memetic, to protect canine hearts with spontaneous cardiac hypertrophy (n=6). *p<0.05 *vs.* baseline. #p<0.05 *vs.* normal hearts (n=5).

an initial sharp decline in myocardial ATP within 30 minutes and plateaued up to 60 minutes of global ischemia. During reperfusion period, both myocardial ATP and heart performance remained depressed (20% of normal baseline, p<0.05). Following 60 minutes of aortic cross clamping and reperfusion, on pump, global myocardial necrosis was negligible. There were no pieces of evidence of necrosis was observed in the group undergone 6 episodes of 10 minutes of warm aortic cross clamping and 10 minutes reperfusion followed by 60 minutes sustained reperfusion suggesting that recovery of left ventricular function, is a crucial primary end-point in the experimental and clinical settings. It is plausible that myocardial stunning, per se, represents an adaptive response and reflecting a mechanism of self-preservation for survival.

Adenosine preconditioning against myocardial stunning in canine model of spontaneous cardiac hypertrophy

Adenosine theory was introduced as a possible mechanism of ischemic preconditioning-mediated cardioprotection [36-40]. At the onset of global or regional myocardial ischemia, adenosine is released from myocardial cell activating adenosine receptors stimulating signal pathway leading to preconditioning cardioprotective effects. Indeed, adenosine has been reported to trigger ischemic preconditioning in healthy animal models with naïve heart; however, its cardioprotective effects in animal models with chronic compensated ventricular hypertrophy have not been investigated.

Normal control group (n=5) and spontaneously hypertensive (n=6) dogs with spontaneous cardiac hypertrophy (wall thickness 33.5 ± 3 mm), on cardiopulmonary bypass, were instrumented to monitor left ventricular performance determined from the relationship between Stroke Work/Diastolic Length (SW/EDL Slope) using sonomicrometry and left ventricular balloon-tipped catheter (Millar). After 30 minutes of stabilization on-bypass pump, hearts were subjected to 30 minutes of normothermic global ischemia and 120 min reperfusion. Tissue biopsies were obtained before, during and after global ischemia for ATP pool analysis. Three boluses of vehicle solution (Control group n=8) or adenosine (100 μM), without adenosine deaminase inhibitors, (n=6) was infused into the CPB reservoir at: a) baseline, b) 5 min before aortic cross-clamping and c) 5 minutes before reperfusion. Adenosine failed to cardioprotect hypertrophic cardiomyopathy hearts against reversible postischemic left ventricular dysfunction "stunning" associated with aortic cross-clamping during cardiac surgery (Table 1).

Acute myocardial infarction triggers ischemic preconditioning in rabbit hearts

A brief episode of ischemic preconditioning is reported to limit infarct size after an acute MI challenge in normal, but not diseased hearts including acutely infarcted hearts. The following experimental protocol was designed to determine whether a primary MI induced by acute sustained sublethal coronary artery occlusion and reperfusion produces cardioprotection in the same or in a remote vascular bed undergoing a secondary acute MI challenge. We also determined whether mitochondrial ATP-sensitive potassium (mito-K-ATP) channels are involved MI-induce preconditioning, a selective blocker of the mito-K-ATP channel, 5-hydroxy-decanoate (5-HD, 5 mg/Kg, i.v.) was infused prior to the first MI. Results demonstrated that 30 minutes of LAD, CFX or RCA coronary artery and 60 minutes reperfusion produced about 20% to 25% infract size of the corresponding risk areas (Figure 4a, 4b). The second MI was initiated by occluding the LAD after 10 minutes from the first 30 minutes occlusion (first MI) and reperfused for 60 minutes. The infarct size was not significantly changed when reinfarction was applied to the same vascular bed (LAD) suggesting that prior MI prevented further expansion of infarct size after reinfarction in the same vascular bed.

Those observations were reproducible regardless which left coronary artery (LAD or CFX) was occluded first followed by first or second RCA occlusions. Due to the proximity of the LAD and CFX and possible cross-interactions and possibility of cardiogenic shock, we have chosen not to occlude two coronary arteries in the left anterior and posterior vascular bed to evade cardiogenic shock.

Chronic heart failure induces chronic conditioning in canine model

In normal dogs' hearts, a brief ischemic preconditioning stimulus reduced the infarct size, following 90 minutes LAD occlusion and reperfusion, from 45 ± 5% to 8 ± 4% (Figure 4a). In the absence of ischemic preconditioning, the infarct size in dogs with congestive heart failure after 90 minutes' sustained ischemia and 4 hrs reperfusion was below 18 ± 3% of the risk areas. (Figures 3a-3c) lists the differences between normal and dilated canine hearts with respect to heart performance (Stroke Work/End Diastolic lengths (dyne. Cm 2 × 103), wall thickening, segmental shortening, fractional shortening, ejection fraction (%) and end-diastolic volume. Reproducible results demonstrated that in age-matched normal dogs, the infarct size after 90 minutes of LAD occlusion 4 hrs reperfusion was 32.1 ± 3, (n=8). In separate studies, the infarct size after an acute MI reached 45.4 ± 4% and 55 ± 5 (n=6 and 8). Naïve hearts were more sensitive to an acute myocardial infarction and respond to ischemic preconditioning stimulation by limiting the infarct size (Figure 4a). Based on our early experience that failing hearts are more sensitive to sodium pentobarbital anesthesia and placement on cardiopulmonary bypass, it was assumed that they would be at higher risk for 90 minutes of LAD occlusion and reperfusion than that of normal hearts. Therefore, shorter durations of sustained ischemia of 45 or 60 minutes of LAD occlusion and 4 hrs reperfusion, were first applied (Figure 4b). Surprisingly, results demonstrated that the infarct size in the failing dog hearts was much smaller after 45 minutes (1.95 ± 1.5%) or 60 minutes LAD occlusion (8.50 ± 1.4%) compared to those preconditioned normal dog hearts (21.2 ± 4 and 27 ± 4%). Hence, the duration of LAD occlusion was set at 90 minutes and 4 hrs reperfusion with or without ischemic preconditioning.

The risk areas were comparable between groups (Figure 4b). After 90 min ischemia, the INF size of the naïve hearts averaged 32.1 ± 3%. In other control groups, the infarct size was 45.4 ± 4% and 55 ± 5% (n=5-7). The infarct size in the failing hearts was 19.7 ± 0.7 (p<0.05 compared to that of healthy hearts). The infarct sizes (INF) were lower (2.0 ± 1.5*, 8.5 ± 1.4* and 19.7 ± 0.7*) in the failing hearts than

control groups following 45, 60 and 90 min of ischemia, respectively. One five minutes episode of IPC limited INF size of the naïve hearts but did not affect the small INF size in failing hearts (Figures 4c, 4d).

When ischemic preconditioning was applied to the canine failing heart and subjected to 90 minutes LAD occlusion and reperfusion, the infarct size was not significantly different than un-preconditioned failing hearts 17 ± 3% (Figure 4c). These results confirmed that failing canine hearts are tolerant to an acute MI without ischemic preconditioning maneuver. The differences between infarct size in the normal hearts and dilated hearts was about 27% less infarction in the failing hearts suggesting that diseased hearts are virtually and chronically preconditioned. Results demonstrated that, in the absence of ischemic preconditioning period, the failing dog heart are more tolerant, compared to healthy hearts, to three durations of coronary occlusion (45, 60 and 90 minutes) and 4 hrs reperfusion and that supplementary ischemic preconditioning stimulus failed to add further limitation of the infract size. KATP channel blocker (5-HD) abolished ischemic preconditioning effects in previously normal and in dog heart with dilated cardiomyopathy. It was also interesting to note that selective blockade of KATP channel (5-HD) abolished heart failure-induced preconditioning suggesting that tolerance of the is a result of intrinsic biological adaptation response to chronic rapid pacing-induced dilated cardiomyopathy. Heat shock protein 70 was highly expressed in all failing canine hearts but absent in age-matched normal hearts (Figure 4E).

Ischemic preconditioning in hamster model of idiopathic dilated cardiomyopathy

To avoid chronic stress mediated by chronic rapid pacing, a strain of hamster UN-X 7.1, that develop idiopathic dilated cardiomyopathy with age (about 200 days), were used to determine whether ischemic precondition strategy cardioprotects ageist an acute MI challenge compared to age-matched normal hamsters. In the UM-X 7.1 hamsters, the infarct size after 30 minutes of LAD occlusion and 60 minutes of reperfusion was close to 0%. The infarct size was 8.4 ± 4% and 17 ± 5% after 45 and 60 minutes LAD occlusion, respectively (Figure 5a). Ischemic preconditioning in UM-X 7.1 hamsters did not add additional reduction of the infarct size (p=NS). Pretreatment with a non-selective A1 receptor antagonist SPT or KATP channel blockers abolished anti-infarction properties of idiopathic dilated cardiomyopathy like that of chronic rapid pacing-induced end-stage heart failure in dogs (Figure 5b). ATP pool and HSP70 were determined in separate hearts (Figures 5c,5d).

The role of adenosine receptors and sarcolemma ATP-sensitive potassium channels (KATP) in ischemic preconditioning was determined using a non-selective adenosine receptor antagonist, 8-SPT or as a specific blocker of KATP channels, glibenclamide. Both blockers abolished chronic tolerance of UM-X 7.1 hamster hearts to acute MI.

Expression of HSP 70

Expression of heat shock protein 70 (HSP) 70 was determined as previously described [41]. Figure 5c illustrates expression of the inducible HSP 70 in dialed canine hearts but not in the naïve hearts. Figure 6c demonstrate expression of the constitutive as well as the inducible HSP 70 in dilated hamster hearts, live, kidney and brain.

Discussion

Biological adaptation to stress is not a new phenomenon but it has long been recognized in the field of micro-organisms, plants, animals and humans [16]. In the early seventies, empirical clinical observations were made by cardiologists to suggest that frequent episodes of angina seem to prolong survivability by unknown mechanisms. Basic scientists and clinicians have been fascinated with the observation that angina-mediated improve survivability and attempted to elucidate the mechanisms of myocardial adaptive response in normal canine animal models by repeated episodes of interrupted coronary artery occlusions and reperfusion [1-9]. In a canine model of angina, 4 episodes of 4 min interrupted the circumflex coronary artery occlusion and 4 min reperfusion, in normal dogs, slowed down the rate of ATP depletion in normal dogs [7-9] and limited the infarct size created by 40 minutes of sustained coronary artery occlusion and reperfusion. The fact that 40 minutes circumflex coronary artery used in the initial report was later abundant because it not reproducible due to inadequate ischemic challenge that produced smaller risk and ischemia areas. Later, a prolonged to 90 minutes coronary artery occlusion was adopted as a standard experimental protocol for acute MI challenge. In rabbit and murine models, 30 minutes of the first diagonal of the LAD become a standard sublethal challenge.

The current studies were undertaken to determine the efficacy of classical ischemic preconditioning in five clinically relevant animal models of diseased hearts with acute myocardial stunning, infarction, re-infarction or remote secondary infarction, and models of chronic spontaneous hypertrophic hearts and end-stage heart failure. To pursue these issues, we determined whether 10 minutes global myocardial ischemia and 10 minutes reperfusion can protect the heart against five consecutive episodes of 10 minutes ischemia and 10 minutes reperfusion in a canine model of global warm aortic cross clamping on-pump. As an end-point, heart performance was measured at the end of every 10 minutes reperfusion after each episode of ischemia (stunning). For the first time, we normalized cardiac function recovery at the end of each 10 minutes reperfusion to cardiac function after the first episode of 10 minutes aortic cross clamping and 10 minutes reperfusion after which all subsequent episodes of stunning were compared. In contrast, normalizing recovery of cardia function per baseline values was a serious mistake because ischemic stimulus causes varying degrees of contractile stunning and upon which subsequent stunning is related to the recovery of the first episode of ischemia and reperfusion and not related to baseline values of normal function. It is also plausible that the reversible metabolic and functional compromise, associated with myocardial stunning, per se, is a unique adaptive response aimed at evading metabolic and functional wastage during subsequent episodes of repeated ischemia and reperfusion.

For the last 30 years, it has been postulated that classical ischemic preconditioning was the most powerful modality for protection against myocardial infarction. We have been standing against the assumption that attenuating expansion of myocardial infarction, induced by ischemic preconditioning in previously healthy hearts, is the most powerful cardioprotective modality for three decades. Nevertheless, the ultimate scientific goal should have been investigating augmentation of myocardial conditioning in sick patients and undergoing invasive interventions. Experimental and clinical results have not achieved overwhelming expectations since each patient has different level of conditioning reserve. It is logic to anticipate that the favorable effect of limitation of infarct size should be reflected in concomitant improved hemodynamic stability, global cardiac performance and ultimately quality of live and survival.

The second arm of our investigation was to determine whether adenosine, an IPC memetic that reduced infarct size in the absence of ischemic stimulation, attenuates myocardial stunning in a canine model of spontaneous cardiac hypertrophied cardiomyopathy. Results demonstrated that adenosine, a chemical preconditioning trigger in normal animal and heats, failed to protect against global myocardial stunning in a model of hypertrophied cardiomyopathy, on pump. The third arm of our investigation was designed to determine whether classical ischemic preconditioning augments myocardial tolerance in acutely infarcted rabbit heart, compared to previously non-infarcted normal hearts. Surprisingly, results demonstrated that an acute MI, per se, is also a strong trigger of ischemic preconditioning against re-infarction in the same vascular bed. This was evident by the fact that the infarct size was not enlarged despite of after the second MI in the same vascular bed. MI also reduced the infarct size in a remote virgin vascular bed after the second MI. Ischemic preconditioning stimulus in the virgin remote area further reduced the infract size compared to non-preconditioned group, suggesting that the remote vascular bed was first preconditioned by the first MI, yet, it exerts an adequate remaining ischemic preconditioning reserve (Figures 1, 2b). Results demonstrated that 30 minutes of LAD, CFX or RCA coronary artery and 60 minutes reperfusion produced about 20% to 25% infract size of the corresponding risk areas. The second MI was initiated by occluding the LAD after 10 minutes from the first 30 minutes occlusion (first MI) and reperfused for 60 minutes. The infarct size was not significantly changed when reinfarction was applied to the same vascular bed (LAD) suggesting that prior MI prevented further expansion of infarct size after reinfarction in the same vascular bed.

It is fascinating to observe that a prior acute MI significantly reduced the infarct size after a secondary remote MI. Furthermore, a brief ischemic preconditioning of a remote virgin vascular bed, after the first MI, triggered more powerful reduction in the infarct size of the remote vascular bed. Those observations were reproducible regardless which left coronary artery (LAD or CFX) was occluded first followed by first or second RCA occlusions. Two consecutive occlusions in LAD and CFX beds were avoided to evade cardiogenic shock. Results support the notion that not only brief ischemic trigger, but also pre-existing sublethal sustained ischemia (MI) produces a strong remote myocardial conditioning. Mechanisms of MI-triggered adaptation could be related to stronger and sustained oxidative stress induced by prolonged sublethal coronary artery occlusion and reperfusion promoting myocardial conditioning in the same and in a remote vascular bed. It should be noted that signaling mechanisms of myocardial conditioning in stunned, infarcted and failing hearts was not investigated in the current studies because our main findings were not previously reported and the need for re-evaluation of previous published reports on classical preconditioning in health and disease. Furthermore, despite of numerous reports on signaling mechanisms of classical and remote ischemic preconditioning and they have not led to fully understand the mechanisms by which brief ischemic stimulus induces adaptive responses to withstand an acute MI challenge. Early in 1993, we questioned whether classical ischemic preconditioning is model or a phenomenon? Scientific discrepancies and clinical disappointments associated with applying classical and remote conditioning to diseased patients has been honestly admitted [13].

Our results provide possible explanations of poor clinical outcomes by the virtue that diseased hearts are acutely or chronically stressed and fully preconditioned with exhausted or reduced myocardial conditioning reserve and cannot be additionally benefited from supplementary classical or remote preconditioning ischemic stimuli. Furthermore, our results demonstrated that one sustained episode of sublethal index ischemia of 30 minutes of LAD occlusion and reperfusion producing myocardial infarction, per se, is also a powerful trigger of preconditioning against a second MI. It plausible that mechanisms of adaptation, induced by classical ischemic preconditioning, myocardial stunning or infarction, share oxidative stress signaling induced in previously normal hearts [16,42]. Augmenting myocardial tolerance in patients with advanced stage of cardiac pathology and undergoing invasive interventions is an important clinical challenge. In 1993, we obtained an RO1 entitled "Cardioprotection of the Failing Hearts." We tested whether classical ischemic preconditioning stimuli would provide supplemental tolerance to further injury associated with surgical interventions. Therefore, the fourth arm study was designed to determine the feasibility of enhancing myocardial tolerance in hearts with chronic congestive failure by ischemic preconditioning in two distinct animal models: 1) a canine model of heart failure created by chronic rapid ventricular pacing for 6-8 weeks, and 2) UM-X 7.1 hamsters model that develop idiopathic dilated cardiomyopathy within 200 ± 20 days of age.

Biological explanation for the adaptive response "MEMORY" to ischemic stress and disease

Mechanisms of adaptation of failing hearts, whether created by chronic rapid pacing in dogs or in UM-X 7.1 hamster strain, could be related to chronic oxidative stress-mediated reservation of ATP and expression of HSP 70, ATP. Significant adenosine elevation with ischemic time in hamsters plus rapid heart rate.

When the same vessel was subjected to two sustained sublethal coronary artery occlusions, separated by only 10 minutes of reperfusion period, no additional expansion of infarct size was observed (p=NS, n=5). It is plausible that the vascular bed MI reduced the impact or a secondary MI possibly by MI-induced adaptive tolerance to reinfarction. In contrast, left coronary (LAD or CFX) acute MI induced cardioprotection to a remote MI (RCA). A classical ischemic preconditioning stimulus has been applied to the virgin remote coronary artery after the first MI significantly reduced the infarct size to 1% to 2%, compared to the group that was not subjected to classical ischemic preconditioning ($p<0.001$). These observations may suggest that despite of prior acute MI, the remote virgin vascular bed exhibits apparently adequate preconditioning reserve and was able to further augment infarct size limitation and partial cardioprotection. Furthermore, blockade of the mitochondrial KATP channel abolished MI and subsequent ischemic preconditioning trigger effects, suggesting that the reduced infarct size after MI in previously infarcted hearts is a biological response and is not experimental outlier.

Taking together all the results, it is evident that acute ischemia and reperfusion, that is usually causes myocardial stunning or infarction, provided adaptive responses to tolerate either additional episodes of stunning or myocardial infarction. In addition, stunned, infarcted and failing hearts are chronically experiencing stressful conditions and they are fully conditioned during the initiation and progression of ventricular remodeling and failure, regardless of the etiology oxidative stress. Therefore, diseased hearts deliberately slow-down their functional (*via* stunning and hibernation) and metabolic responses (*via* preservation of the remaining ATP pool and reduced

acidosis) as adaptive responses toward unanticipated increased demand to survive further ischemic challenges [16]. Furthermore, chronic stress in the diseased hearts apparently promotes maximal stimulation molecular signaling mechanisms of survival than normal hearts. These observations might shed some light in explaining the discrepancies and disappointing outcomes of clinical trials and experimental investigation in sick animals and stunned, infarcted and failing hearts [13].

It was interesting to observe that blockade of the mitochondrial KATP channel abolished both classical ischemic preconditioning in normal hearts, as well as, MI-induced remote ischemic preconditioning. It plausible that acquiring and progression of a disease impose acute and chronic stress that drive sick hearts to adapt to comorbid pathologic abnormalities making them more resilient to ischemic stress and these properties could be abolished by of KATP channel blockers. The clinical ramifications for these results should be seriously considered. Sulfonylurea derivatives of KATP channel blockers, frequently used as medications for cardiac diabetic patients could be contraindicated.

Unlike naïve hearts, canine hearts with severe congestive heart failure are tolerant to 45, 60 and 90 minutes acute MI. UM-X 7.1 hamster hearts were tolerant to 30, 45 and 60 minutes of acute MI. Increased tolerance of failing hearts was associated with expression of inducible HSP 70. Glibenclamide abolished chronic tolerance to acute MI in non-preconditioned UM-X 7.1 strain. Ischemic preconditioning did not have any supplemental limitation of the infarct size over that of non-ischemically hearts.

Our results provide a novel basis for myocardial conditioning in clinically relevant diseased hearts and shed lights on needed explanations of poor experimental and clinical outcomes. By the virtue of our novel findings, we conclude that diseased hearts are acutely or chronically stressed, fully preconditioned against MI, exhausted conditioning reserve and cannot be additionally benefited by supplementary classical or remote preconditioning ischemic stimuli. Subjecting sick patients to unnecessary preconditioning maneuvers or using sulfonylureas derivatives could adversely the outcome of medical or surgical coronary interventions.

Limitations

Although, clinically relevant animal models were used in the current studies, they provide an important preclinical finding to differentiate between health and disease in terms of adaptability to short or prolonged ischemic stress. It is fascinating to demonstrate that hearts in advanced stage of disease are adapted to disease-mediated stress and modulate their metabolic and heart performance to evade unanticipated stronger or prolonged stress by slowing down metabolic and contractile reserves to ensure survival despite of being sick. The current studies might be an eye opener to clinicians who have adapted ischemic preconditioning protocols without understanding that disease hearts are trying to protect and prolong their biologic survival by modifying their cellular and molecular response to more stressful events. It is also important to understand that diseased organs are clever and are not a naïve as normal organ. Furthermore, our results warn against the use of anti-diabetic sulfonylureas in patients with ischemic syndromes and heart failure. There are clear pieces of evidence to suggest that these types of medications abolish the acquired disease-mediated adaptability to ischemic stress making patients more vulnerable to morbidity and mortality.

Summary

Results demonstrate that failing hearts were more tolerant to MI ($p<0.05$ *vs.* control) induced by LAD occlusion and reperfusion and that supplemental preconditioning stimuli did not add favorable benefits. Failing hearts are chronically preconditioned against an acute MI and apparently have exhausted their IPC reserve for supplementary conditioning. Sulfonylurea derivative medications abolished myocardial tolerance in diseased heart thus jeopardizes patients' safety and mortality. In pursuing investigation on ischemic preconditioning in diseased hearts, results revealed that not only brief ischemic triggers can produce anti-infarction protection, but also prolonged coronary artery occlusion and reperfusion produce enough stress to fully precondition hearts to evade unanticipated events that might lead to myocardial infarction, cardiac dysfunction and death.

Conclusion

Classical ischemic pre- or remote conditioning, per se, is effective in normal subjects and hearts, but not in diseased subjects. The scientific illusion and delusion that brief ischemic stimuli trigger unlimited mechanisms of adaptation and become the most powerful cardioprotective modality against myocardial infarction has been shattered with disappointing outcomes of clinical trials, without clear explanations. The current investigations provided most needed information explain why classical ischemic preconditioning does not provide supplemental cardioprotection in diseased hearts and subjects. Our results support the discovery that diseased hearts (stunned, infarcted, hypertrophied and failing hearts) utilize maximal anti-infarction conditioning reserve and that supplementary ischemic preconditioning triggers or sulfonylurea medication adversely affect patient's safety.

Clinical Significance

Patients with variable degrees of disease exhibit underlying stress-related adaptive responses to evade anticipated stronger ischemic challenge. Therefore, additional stimuli (ischemic, hypoxic or chemical and maneuvers) directed toward promoting supplemental cardioprotection might cause more harm than protection. Drugs known to negatively influence anti-infarction mechanisms induced by ischemic preconditioning, i.e., sulfonylurea derivatives, must be avoided in diabetics undergoing transcatheter endovascular interventions or invasive surgical repairs.

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