Published in final edited form as:

J Cardiovasc Pharmacol. 2010 April ; 55(4): 408–416. doi:10.1097/FJC.0b013e3181d4ca66.

Caspase Inhibition Modulates Left Ventricular Remodeling Following Myocardial Infarction through Cellular and Extracellular Mechanisms

William M. Yarbrough, Rupak Mukherjee, Robert E. Stroud, Evan C. Meyer, G. Patricia Escobar, Jeffrey A. Sample, Jennifer W. Hendrick, Joseph T. Mingoia, and Francis G. Spinale

Division of Cardiothoracic Surgery Medical University of South Carolina, Charleston, South Carolina, Ralph H. Johnson Veterans' Association Medical Center, Charleston, South Carolina

Abstract

Background—Myocyte death occurs by necrosis and caspase-mediated apoptosis in myocardial infarction (MI). *In-vitro* studies suggest caspase activation causes myocardial contractile protein degradation without inducing apoptosis. Thus, caspase activation may evoke left ventricular (LV) remodeling through independent processes post-MI. The effects of caspase activation on LV geometry post-MI remain unclear. This project applied pharmacologic caspase inhibition (CASPI) to a porcine model of MI.

Methods and Results—Pigs (34kg) were instrumented in order to induce 60min of coronary artery occlusion followed by reperfusion and a 7-day follow-up period. Upon reperfusion, the pigs were randomized to saline (n=12) or CASPI (n=10, IDN6734, 6mg/kg IV, then 6mg/kg/hr for 24hrs). Plasma troponin-I values were reduced with CASPI compared to saline at 24hrs post-MI (133 ± 15 vs 189 ± 20 ng/mL, respectively, $p < 0.05$). LV end-diastolic area (echocardiography) and inter-regional length (sonomicrometry) increased from baseline in both groups but were attenuated with CASPI by 40% and 90%, respectively ($p < 0.05$). Myocyte length was reduced with CASPI compared to saline (128 ± 3 vs 141 ± 4 μ m, respectively, $p < 0.05$). Plasma free-pro-MMP-2 values increased from baseline with CASPI ($27 \pm 6\%$, $p < 0.05$) indicative of reduced conversion to active MMP-2. Separate *in-vitro* studies demonstrated that activated caspase species cleaved pro-MMP-2 yielding active MMP-2 forms and that MMP activity was increased in the presence of activated caspase-3.

Conclusion—CASPI attenuated regional and global LV remodeling post-MI and altered viable myocyte geometry. Caspases increased MMP activity *in-vitro* while CASPI modified conversion of MMP-2 to the active form *in-vivo*. Taken together, the results of the present study suggest that the elaboration of caspases post-MI likely contribute to LV remodeling through both cellular and extracellular mechanisms.

Keywords

myocardial infarction; remodeling; caspase inhibition; left ventricular

Address for Correspondence: Francis G. Spinale, MD, PhD Cardiothoracic Research 770 MUSC Complex, STRB, Suite 625 Medical University of South Carolina Charleston, South Carolina 29425 Phone: 843-876-5186, Fax: 843-876-5187.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

INTRODUCTION

Prolonged myocardial ischemia and/or infarction (MI) may alter the geometry and structural characteristics of the left ventricular (LV) myocardium [1]. These changes, collectively termed “LV remodeling,” encompass dynamic interactions within both cellular and extracellular myocardial compartments. Historically, myocytes that succumbed to prolonged ischemia were believed to have progressed through stages of reversible and irreversible injury followed by necrosis [2–3]. However, “programmed cell death,” or apoptosis, has recently been described as a potentially important contributing entity in the progression and evolution of both MI and subsequent LV remodeling processes [4–5]. The caspases are an endogenous family of intracellular cysteine proteases that facilitate execution of apoptotic cascades, particularly in the setting of myocardial ischemia and MI [3]. While past studies provide a cause-effect relationship between caspase activity and myocyte injury [6–9] and apoptosis [7–10], an *in-vitro* investigation suggests that multiple substrates for caspase activity may exist [11]. Importantly, these intracellular substrates may be proteolytically processed independent of a cellular commitment to apoptosis [11]. Thus, caspase activation may evoke LV remodeling post-MI through two independent processes that incorporate either apoptotic or non-apoptotic mechanisms. However, the design and execution of a systematic study examining the effects of early caspase inhibition upon LV remodeling in an animal model of MI that recapitulates clinical contexts remains to be performed. Moreover, a direct association connecting caspase activity with determinants of myocardial extracellular matrix remodeling remains to be defined. Accordingly, the goal of the present study was to examine the effects of broad-spectrum caspase inhibition upon LV remodeling processes and to establish a link between potential cellular and extracellular mechanisms responsible for defining the structural characteristics of the LV myocardium following MI.

METHODS

Study Rational / Overview

This study employed a chronic porcine model in which the effects of pharmacologic broad-spectrum caspase inhibition (CASPI) upon indices of regional and global LV remodeling were assessed following MI. CASPI was instituted immediately prior to myocardial reperfusion and for a finite period of time in order to simulate the clinical context. All animals were treated and cared for in accordance with the National Institute of Health “Guide for the Care and Use of Laboratory Animals (National Research Council, Washington, 1996).”

Experimental Design

Instrumentation—Yorkshire pigs (n=22, 34 kg, Hambone Farms, Orangeburg, SC) were anesthetized with isoflurane (3%/1.5L/min) and nitrous oxide (0.5L/min). A thoracotomy was performed and vascular access catheters (7-Fr) were sutured into the descending thoracic aorta and pulmonary artery. A disengaged coronary snare and piezoelectric crystals (2mm, Sonometrics, Ontario) were positioned as demonstrated in Figure 1. Opposing ends of the crystal cables coalesced into a single connector hub that exited the chest and was buried subcutaneously with the distal end of the snare.

Randomization—Five days following instrumentation the pigs were randomized in blinded fashion to saline or CASPI as shown in Figure 2. Eleven normal, age-matched, non-instrumented pigs were included for comparison.

Baseline Measurements—The pigs were sedated with diazepam (200mg-PO, Barr Laboratories, Pomona, NY) and echocardiographic images (ATL Ultramark VI, 2.25MHz transducer, Bothell, WA) were obtained as previously described [12–14]. The vascular access

catheters were used to obtain plasma for subsequent troponin-I determination, transduce arterial pressure, and infuse heparin (150U/kg-IV, Pharmacia and Upjohn, Kalamazoo, MI). Sonomicrometry crystals were used to assess distances between the remote and area-at-risk regions.

MI Protocol—Snare tightening produced MI confirmed by electrocardiographic and hemodynamic changes. After 60min of ischemia, the snares were disengaged to allow reperfusion. CASPI was instituted immediately prior to reperfusion and was achieved with a bolus of IDN6734 (Idun Pharmaceuticals, San Diego California, 6mg/kg-IV) followed by a 24hr infusion (6mg/kg/hr-IV) through the pulmonary artery catheter. This dosing regimen achieved desired plasma concentrations of IDN6734 (790 ± 142 ng/mL) as predicted by prior pharmacokinetic studies. Plasma troponin-I levels were assessed 24hrs post-MI using the AxSYM automated microparticle enzyme immunoassay analyzer (Abbott Laboratories, Abbott Park, IL). At 7 days post-MI, echocardiographic and sonomicrometry crystal measurements were repeated and a LV instrumentation catheter study was performed as previously described [15].

Myocardial and Plasma Interrogation—The LV was explanted and infarct size was estimated as a percentage of the area-at-risk using tetrazolium chloride staining [16]. Plasma free-pro-MMP-2 levels were quantified using a human MMP-2 Biotrak ELISA system (Amersham Biosciences Corp, Piscataway, NJ). MMP activity was determined by fluorogenic substrate analysis as described previously [17]. Caspase-3 activity was determined in remote LV regions using a RediPlate 96 EnzChek Kit (Molecular Probes, Inc., Eugene, OR). Contractile protein (actin, myosin, and troponin-I, C, and T) abundances [18] and collagen content [19] were determined within remote, border, and infarcted regions of the LV using modifications of protocols previously described by this laboratory. Myocyte dimensions were determined from cells isolated from formalin-fixed remote LV regions utilizing potassium-hydroxide digestion (50 myocytes/region/pig) as described previously [20].

Caspases and MMP Activity *In-Vitro*—Homogenized LV tissue from control pigs (20 μ g), purified fluorogenic substrate for MMP-1, 2, 3, 7, and 9 (Calbiochem), and activated caspase-3 (50ng/mL, BD Biosciences) were prepared and combined with and without the non-specific and irreversible caspase inhibitor, z-VAD (z-Val-Ala-Asp-fluoromethyl-ketone, 250 μ M, Sigma), to assess the effects of caspase-3 upon *in-vitro* MMP activity. An additional *in-vitro* study examined the effects of increasing concentrations of activated caspases 1–10 (BioVision K233-10-25) on uniform concentrations of recombinant human pro-MMP-2 (R&D Systems, 902-MP). Standards for MMP-2 and –9 (Chemicon, CC073) were used for molecular weight comparison.

Data Analysis—Changes in hemodynamic, functional, and geometric values between the saline and CASPI groups were analyzed using a two-way analysis of variance (ANOVA) followed by mean separation using pair-wise Bonferroni corrections. Values are presented as the mean and standard error of the mean and p-values of less than 0.05 were considered statistically significant.

RESULTS

External defibrillation for ventricular fibrillation was required in 18% of the pigs during ischemia with no difference between groups (Pearson Corrected Chi-Square analysis, $p > 0.97$). There were no episodes of ventricular fibrillation after reperfusion.

Myocardial Injury and Steady-State Hemodynamics

Values for infarct size, plasma troponin-I, and steady-state hemodynamics are summarized in Table 1. CASPI was associated with a significant reduction in troponin-I release at 24hrs post-MI compared to saline values. However, this difference was not apparent 72hrs post-MI and failed to translate into a significant reduction in infarct size at 7 days post-MI ($p=0.42$). There were no significant differences in heart rate, cardiac output, or mean pulmonary artery pressure between the control and MI groups. LV end-diastolic pressure was increased in both MI groups compared to control values. However, compared to control, mean aortic and LV peak pressure was decreased in the CASPI group.

LV Geometry and Regional Stress

Echocardiography and inter-regional distances, or chord lengths, were used to assess global and regional LV geometry, respectively (Figure 3). At 7 days post-MI, LV area was increased in both MI groups compared to baseline values. However, compared to saline values, LV area was significantly attenuated with CASPI. Inter-regional chord length was increased in both MI groups compared to baseline but was significantly reduced with CASPI compared to saline values. Septal and posterolateral wall stress increased in both MI groups compared to control values. However, posterolateral wall stress was significantly attenuated with CASPI.

Myocyte and Extracellular Matrix Remodeling

Values for myocyte dimensions and regional collagen content are presented in Table 1. Myocyte length was significantly decreased in the CASPI group compared to saline values and myocyte width was significantly reduced in both MI groups compared to control values. A uniform region-specific increase in collagen content progressing from the remote region towards the infarct was observed in both MI groups. Contractile protein abundances were determined within the LV as well (Figure 4). A region-specific decrease in the abundance of contractile proteins was observed in both MI groups that progressed from remote regions towards the infarct.

Plasma MMP Assay and LV Caspase-3 Activity

Plasma-pro-MMP-2 was assessed at baseline and following reperfusion (Figure 5). At 30min post-reperfusion, plasma-pro-MMP-2 was similarly decreased in both MI groups compared to baseline values. However, by 24hrs post-MI, plasma-pro-MMP-2 levels appeared to have normalized in the saline group. In contrast, a marked increase in the abundance of plasma-pro-MMP-2 was observed in the CASPI group compared to both baseline and saline values. Caspase-3 activity was determined within the remote regions of the LV at 7 days post-MI (Figure 6). Similar degrees of protease activity were observed in the control and saline groups. However, protease activity was significantly reduced in pigs randomized to CASPI.

Caspase-Induced MMP Activity

The effects of activated caspase-3 on MMP activity were assessed *in-vitro* using a purified fluorogenic substrate for MMP-1, 2, 3, 7, and 9 and LV tissue collected from control pigs (Figure 7). MMP activity was significantly increased compared to control values (LV tissue + substrate) in the presence of activated caspase-3. However, this increase was abolished by the addition of the caspase inhibitor z-VAD. The effects of increasing concentrations of activated caspases 1–10 on uniform concentrations of recombinant human pro-MMP-2 were assessed *in-vitro* as well. Zymography demonstrated that the activated caspases cleaved the pro-form of MMP-2 (Figure 8). Moreover, increasing concentrations of activated caspases were associated with further degradation of the initial MMP-2 cleavage product.

DISCUSSION

Left ventricular (LV) remodeling is an inevitable structural consequence of myocardial infarction (MI) and an independent predictor of morbidity and mortality post-MI [21]. While the precise etiology of MI-induced LV remodeling remains unclear, alterations within both the cellular and extracellular myocardial compartments likely contribute to the initiation and progression of this maladaptive process. Past studies have clearly established that matrix metalloproteinase (MMP) activation facilitates the progression of adverse LV remodeling post-MI [12–14,22–23]. Activation of MMPs may be accomplished through multiple upstream proteolytic cellular cascades, some of which remain unknown [1]. It remains to be established whether a relationship exists between caspase activation and MMP activity in the context of MI. In an attempt to address these issues, broad-spectrum caspase inhibition was employed in a well-defined porcine model of MI [12–14]. The unique findings of the present study were two-fold. First, caspase inhibition attenuated regional and global LV remodeling post-MI and was associated with alterations in viable myocyte geometry. Second, caspase activity evoked increases in MMP activity *in vitro* while early caspase inhibition modified conversion of MMPs to their active forms *in vivo*. Taken together, the results of the present study suggest that the elaboration of caspases post-MI likely contributed to LV remodeling through both cellular and extracellular mechanisms.

Caspases and Myocardial Ischemia

The present study builds upon the results of these past investigations by examining the effects of caspase inhibition on myocardial injury and regional and global LV remodeling in a large animal model. Moreover, caspase inhibition was deployed in an experimental scenario that recapitulated the clinical context. Specifically, caspase inhibition was instituted at the time of reperfusion and was continued for a period of 24hrs. The present study is unique in that it demonstrated that a finite period of caspase inhibition significantly attenuated regional and global LV remodeling beyond the acute-MI period. Importantly, these results extend the observations of past studies through the suggestion that caspase activation plays multiple roles in the structural dynamics on the LV myocardium post-MI.

LV Regional and Global Geometry Post-MI

Broad-spectrum caspase inhibition was associated with significant reductions in two independent assessments of LV remodeling post-MI, namely, LV chamber area and LV inter-regional distance. The reduction in LV dilation conferred by caspase inhibition during resolution of the acute MI period translated into significant reductions in posterolateral wall stress. A past *in vitro* study utilizing papillary muscles suggested that increases in stretch could evoke apoptosis [24]. Therefore, the reduction in wall stress associated with caspase inhibition in the present study may have in turn resulted in decreased activation of intracellular caspase cascades as well as other proteolytic systems during the post-MI period. However, it must be recognized that the significant attenuations in LV remodeling achieved by caspase inhibition did not translate into improvements in LV pump function at 7 days post-MI. Although the etiology for this functional effect is multi-factorial, it is likely that the duration of the experimental protocol played a role. Had the protocol been significantly lengthened, a beneficial effect on LV pump function may have been observed.

Cellular Events and Remodeling Post-MI

In the present study, marked elevations in plasma troponin-I values were observed at 24hrs post-MI in both MI groups indicating that significant degrees of myocyte loss were inflicted by the coronary occlusion technique. Caspase inhibition was associated with a significant decrease in plasma troponin-I values at 24hrs post-MI compared to the saline group. However, this difference was not apparent at 72hrs post-MI and did not translate into a significant

reduction in infarct size at 7 days post-MI. These results may have been due to the finite period of caspase inhibition employed in the present study. Importantly, the observed reduction in troponin-I values paralleled the duration of caspase inhibition used in this study. Thus, it is likely that caspase inhibition succeeded in altering the extent of myocardial injury to some degree during the acute MI period. Moreover, the transient reduction in plasma troponin-I values conferred by caspase inhibition is a result that is not dissimilar to those of observed in past studies in which early modulation of caspase activity attenuated myocardial injury following myocardial ischemia and reperfusion [6–9]. Taken together, these results suggest that modulation of caspase activity, if even for only a finite period of time, can achieve beneficial effects on regional and global LV remodeling post-MI independent of myocardial viability as assessed by absolute infarct size. However, it must be noted that a direct assessment of myocardial apoptosis was not performed in this study. Apoptosis has been previously demonstrated to peak approximately 12 hrs following the onset of myocardial ischemia [25] and therefore was not evaluated in this 7-day post-MI study. Nevertheless, an attempt was made to quantify caspase activity within the remote regions following terminal measurements. Caspase inhibition was associated with a significant reduction in caspase-3 activity within the remote region compared to the saline group suggesting that a pharmacologic effect was achieved with the dosing regimen that was employed. While remaining speculative, it is likely that apoptotic rates were reduced by caspase inhibition in the same regions as well. The pharmacologic effect demonstrated by the caspase activity assay had significant effects on viable myocyte geometry, an important determinant of LV geometry post-MI. Specifically, caspase inhibition was associated with a significant decrease in myocyte length post-MI. Importantly, the decrease in myocytes length conferred by caspase inhibition likely reduced myocytes volume and contributed to the attenuations in LV dilation. Caspase inhibition also appeared to reduce total contractile protein abundance post-MI. This reduction was likely a reflection of the smaller myocyte lengths associated with this group. The observed decreases in the relative abundances of the lower molecular weight sub-fractions of troponin-T may have been indicative of attenuations in contractile protein degradation associated with caspase inhibition. Whether and to what degree these effects on contractile protein would eventually alter LV contractility remains to be determined.

Matrix Remodeling Post-MI

The present study sought to determine whether and to what degree modulation of caspase activity would influence a distinct proteolytic system known to facilitate LV dilation post-MI, the MMPs. The present study includes several independent findings that suggest that an interaction exists between caspase activity in the post-MI state and the activation of MMPs. First, *in-vivo* administration of CASPI in the post-MI period significantly increased plasma levels of pro-MMP-2, which would suggest reduced conversion to the active form of MMP-2. Second, *in-vitro* measurements in LV myocardial homogenates demonstrated that inhibition of active caspases, will in turn reduce total MMP proteolytic activity. Third, using a purified recombinant system, it was demonstrated that active caspases can directly convert pro-MMP-2 to an active form of MMP-2. Taken together, these findings suggest that caspase induction and activation, which has been demonstrated to occur within the post-MI period, can directly influence extracellular matrix remodeling processes, through proteolytic processing and activation of MMPs. While the *in-vivo* and *in-vitro* studies described in the investigation remain associative, these observations put forth the intriguing postulate that the interstitial release of active caspases during the final stages of apoptosis can in turn alter the local extracellular environment, which in turn would promulgate both adverse cellular and extracellular remodeling in the post-MI period.

The observed increase in pro-MMP-2 plasma levels associated with CASPI in the post-MI period is particularly noteworthy. The observed increase may have been due to increased

transcription and synthesis of MMP-2, reduced post-translational processing through reduced conversion and activation of MMP-2, or a combination of these determinants. The results from the present study, as well as from past reports [26], suggest that changes in pro-MMP-2 levels were due in large part, to reduced post-translational processing. Specifically, using *ex-vivo* and *in-vitro* assays, the present study directly demonstrated that active caspases can convert pro-MMP-2 to the active form. In a past clinical study, it was demonstrated that through the measurement of myocardial gradients of pro-MMP-2, that increased conversion to the active form of MMP-2 occurred following ischemia-reperfusion [26]. However, in the present study increased levels of pro-MMP-2 were observed following a period of chronic CASPI administration. Thus, from a temporal perspective, a contributory factor for the increased plasma levels of pro-MMP-2 could be due to CASPI altering transcriptional and synthesis rates. Based upon the findings from the present study, this issue warrants future investigation.

Because MMPs are active within the myocardial interstitium within minutes of the ischemic onset [27], the observed conversion in the relative abundance of active-MMP-2 likely contributed to the beneficial effects of caspase inhibition on LV remodeling post-MI. The observed shift in the abundance of active-MMP-2 did not result in significant differences in absolute myocardial collagen content suggesting that caspase inhibition did not significantly alter myocardial wound healing. Analogous to past studies in which MMP inhibition was employed in experimental models of MI [12–14], the beneficial effects of caspase inhibition on LV remodeling observed in the present study did not translate into improvements in global pump function. Thus, both MMP and broad-spectrum caspase inhibition may have reduced LV dilation post-MI through common extracellular mechanisms.

Study Limitations and Clinical Implications

In the present study, caspase inhibition was associated with a reduction in mean arterial pressure. A similar reduction in arterial pressure was observed in a past study that utilized caspase inhibition in the setting of myocardial ischemia and reperfusion [28]. Although the significance and precise mechanism responsible for this systemic effect remains to be determined, it is likely that modulation of caspase activity influenced systemic vascular resistance properties. Importantly, this result was not associated with adverse effects on LV pump function as assessed by cardiac output.

The effects of caspase inhibition in the setting of normal ambient conditions were not assessed in this investigation. In the absence of a pathological stimulus, the induction of caspases and in turn apoptosis rates within the myocardium is likely to be quite low. Indeed, a recent study identified that active caspases are at extremely low levels within the normal myocardium [29]. Based upon the findings from the present study, this would suggest that caspase mediated activation of myocardial MMPs would be extremely low under normal, ambient conditions. Thus, while remaining speculative, this would imply that CASPI treatment in normal, non-MI preparations would have minimal effects on LV structure and function. However, this was not directly addressed in the present study design, and warrants future study.

The results of the present study outline new directions for continued efforts to define the role of caspases in the LV myocardium post-MI. Issues of particular importance include a necessity to define the effects of caspase inhibition on LV contractility and to elucidate additional cellular cascades with which caspases may interact post-MI. A recent *in vitro* investigation by Laplante and associates suggests that that caspases can alter the extracellular environment [30]. Specifically, these investigators demonstrated that connective tissue growth factor release can be mediated by caspases. Importantly, the present study suggests that a link exists between the caspase and matrix metalloproteinase proteolytic systems within the post-infarction myocardium. While inhibition of caspases and matrix metalloproteinases has yet to achieve clinical pharmacological relevance, continued investigations aimed at targeting and inhibiting

specific species of these proteolytic enzymes may not only prove to be efficacious, but may also eliminate deleterious side effects associated with broad-spectrum inhibition. Moreover, the results of the present study suggest that caspases are involved in multiple functions in the myocardium post-MI. Thus, these results may extend the potential role of pharmacologic caspase inhibition beyond that of myocardial salvage alone.

Acknowledgments

This study was supported by the National Heart, Lung, and Blood Institute Grants HL-87134, HL-81692, a Merit Award from the Ralph H. Johnson Veterans' Association Medical Center, the National Institute of Health Postdoctoral Training Grant HL-07260, and the American Association for Thoracic Surgery's Third Edward D. Churchill Research Scholarship. A basic science research grant was provided by Idun Pharmaceuticals, Inc. The authors acknowledge contributions made by Robert C. Armstrong, Patrick C. White and Ann Stiko in the completion of this manuscript.

REFERENCES

- 1). Creemers EE, Cleutjens JP, Smits JF, et al. Matrix metalloproteinase inhibition after myocardial infarction: a new approach to prevent heart failure? *Circ Res* 2001;89(3):201–10. [PubMed: 11485970]
- 2). Reimer KA, Jennings RB. The “wavefront phenomenon” of myocardial ischemic cell death. II. Transmural progression of necrosis within the framework of ischemic bed size (myocardium at risk) and collateral flow. *Lab Invest* 1979;40(6):633–44. [PubMed: 449273]
- 3). Gill C, Mestral R, Samali A. Losing heart: the role of apoptosis in heart disease--a novel therapeutic target? *FASEB J* 2002;16(2):135–46. [PubMed: 11818361]
- 4). Mani K, Kitsis RN. Myocyte apoptosis: programming ventricular remodeling. *J Am Coll Cardiol* 2003;41(5):761–4. [PubMed: 12628719]
- 5). Abbate A, Biondi-Zoccai GG, Bussani R, et al. Increased myocardial apoptosis in patients with unfavorable left ventricular remodeling and early symptomatic post-infarction heart failure. *J Am Coll Cardiol* 2003;41(5):753–60. [PubMed: 12628718]
- 6). Huang JQ, Radinovic S, Rezaiefar P, et al. In vivo myocardial infarct size reduction by a caspase inhibitor administered after the onset of ischemia. *Eur J Pharmacol* 2000;402(1–2):139–42. [PubMed: 10940367]
- 7). Holly TA, Drincic A, Byun Y, et al. Caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. *J Mol Cell Cardiol* 1999;31(9):1709–15. [PubMed: 10471354]
- 8). Yaoita H, Ogawa K, Maehara K, et al. Attenuation of ischemia/reperfusion injury in rats by a caspase inhibitor. *Circulation* 1998;97(3):276–81. [PubMed: 9462530]
- 9). Kovacs P, Bak I, Szendrei L, et al. Non-specific caspase inhibition reduces infarct size and improves post-ischaemic recovery in isolated ischaemic/reperfused rat hearts. *Naunyn Schmiedeberg's Arch Pharmacol* 2001;364(6):501–7. [PubMed: 11770004]
- 10). Okamura T, Miura T, Takemura G, et al. Effect of caspase inhibitors on myocardial infarct size and myocyte DNA fragmentation in the ischemia-reperfused rat heart. *Cardiovasc Res* 2000;45(3):642–50. [PubMed: 10728385]
- 11). Communal C, Sumandea M, de Tombe P, et al. Functional consequences of caspase activation in cardiac myocytes. *Proc Natl Acad Sci* 2002;99(9):6252–6. [PubMed: 11972044]
- 12). Mukherjee R, Brinsa TA, Dowdy KB, et al. Myocardial infarct expansion and matrix metalloproteinase inhibition. *Circulation* 2003;107(4):618–25. [PubMed: 12566376]
- 13). Yarbrough WM, Mukherjee R, Brinsa TA, et al. Matrix metalloproteinase inhibition modifies left ventricular remodeling after myocardial infarction in pigs. *J Thorac Cardiovasc Surg* 2003;125(3):602–10. [PubMed: 12658202]
- 14). Yarbrough WM, Mukherjee R, Escobar GP, et al. Selective targeting and timing of matrix metalloproteinase inhibition in post-myocardial infarction remodeling. *Circulation*. In press.
- 15). McElmurray JH, Mukherjee R, New RB, et al. Angiotensin-converting enzyme and matrix metalloproteinase inhibition with developing heart failure: comparative effects on left ventricular function and geometry. *J Pharmacol Exp Ther* 1999;291(2):799–811. [PubMed: 10525103]

- 16). Ytrehus K, Liu Y, Tsuchida A, et al. Rat and rabbit heart infarction: effects of anesthesia, perfusate, risk zone, and method of infarct sizing. *Am J Physiol* 1994;267(6 Pt 2):H2383–90. [PubMed: 7528994]
- 17). Coker ML, Thomas CV, Clair MJ, et al. Myocardial matrix metalloproteinase activity and abundance with congestive heart failure. *Am J Physiol* 1998;274(5 Pt 2):H1516–23. [PubMed: 9612358]
- 18). Eble DM, Walker JD, Mukherjee R, et al. Myosin heavy chain synthesis is increased in a rabbit model of heart failure. *Am J Physiol* 1997;272(2 Pt 2):H969–78. [PubMed: 9124461]
- 19). Spinale FG, Zellner JL, Johnson WS, et al. Cellular and extracellular remodeling with the development and recovery from tachycardia-induced cardiomyopathy: changes in fibrillar collagen, myocyte adhesion capacity and proteoglycans. *J Mol Cell Cardiol* 1996;28(8):1591–608. [PubMed: 8877770]
- 20). Gerdes AM, Onodera T, Tamura T, et al. New method to evaluate myocyte remodeling from formalin-fixed biopsy and autopsy material. *J Card Fail* 1998;(4):343–8. [PubMed: 9924856]
- 21). White HD, Norris RM, Brown MA, et al. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation* 1987;76(1):44–51. [PubMed: 3594774]
- 22). Ducharme A, Frantz S, Aikawa M, et al. Targeted deletion of matrix metalloproteinases-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction. *J Clin Invest* 2000;106(1):55–62. [PubMed: 10880048]
- 23). Rohde L, Ducharme A, Arroyo L, et al. Matrix metalloproteinases inhibition attenuates early left ventricular enlargement after experimental myocardial infarction in mice. *Circulation* 1999;99(23):3063–3070. [PubMed: 10368126]
- 24). Cheng W, Li B, Kajstura J, et al. Stretch-induced programmed myocyte cell death. *J Clin Invest* 1995;96(5):2247–59. [PubMed: 7593611]
- 25). Cheng W, Kajstura J, Nitahara JA, et al. Programmed myocyte cell death affects the viable myocardium after infarction in rats. *Exp Cell Res* 1996;226(2):316–27. [PubMed: 8806435]
- 26). Joffs C, Gunasinghe H, Multani MM, et al. Cardiopulmonary bypass induces the synthesis and release of matrix metalloproteinases. *Ann Thorac Surg* 2001;71:1518–23. [PubMed: 11383793]
- 27). Etoh T, Joffs C, Deschamps A, et al. Myocardial and interstitial matrix metalloproteinases activity after acute myocardial infarction in pigs. *Am J Physiol Heart Circ Physiol* 2001;281(3):H987–94. [PubMed: 11514263]
- 28). Minatoguchi S, Kariya T, Uno Y, et al. Caspase-dependent and serine protease-dependent DNA fragmentation of myocytes in the ischemia-reperfused rabbit heart: these inhibitors do not reduce infarct size. *Jpn Circ J* 2001;65(10):907–11. [PubMed: 11665797]
- 29). Park M, Shen Y-T, Gaussin V, et al. Apoptosis predominates in nonmyocytes in heart failure. *Am J Physiol Heart Circ Physiol* Aug;2009 297(2):H785–91. [PubMed: 19465551]
- 30). Laplante P, Sirois I, Raymond MA, et al. Caspase-3-mediated secretion of connective tissue growth factor by apoptotic endothelial cells promotes fibrosis. *Cell Death Differ* Sept 4;2009 :1–13. [PubMed: 19079285]

FIGURE 1.

(LEFT) A schematic of the porcine heart following basic instrumentation. A disengaged snare device was placed around the proximal circumflex coronary artery. Sonomicrometry crystals were positioned within the anterior (remote region) and posterolateral (area-at-risk, *shaded*) LV surfaces. Access ports were sutured into the descending thoracic aorta and pulmonary artery trunk (not shown). (RIGHT) A depiction of the LV as observed from a view perpendicular to the long-axis. Sonomicrometry crystal heads are apparent along the endocardial surface of the LV.

FIGURE 2.

The experimental design included a 5-day period to recover from basic instrumentation. Following collection of baseline data, which included assessment of regional and global LV geometry, the pigs were subjected to 60 min regional myocardial ischemia followed by randomization to saline or CASPI. Terminal measurements obtained at 7 days post-MI included tissue infarct sizing. Previous porcine MI-reperfusion studies utilizing microsphere injections confirmed immediate and total reperfusion of the area-at-risk.

FIGURE 3.

(LEFT) LV end-diastolic and end-systolic areas presented as changes from baseline. At 7 days post-MI, LV areas were increased in both MI groups compared to baseline values. CASPI conferred significant reductions in LV dilation compared to saline values. (RIGHT) At baseline and at 7 days post-MI, end-diastolic and end-systolic inter-regional “chord” lengths were determined between the remote and area-at-risk regions yielding distances that approximated the diameter of the LV. LV end-diastolic and end-systolic chord lengths were increased in the saline group at 7 days post-MI. CASPI attenuated these geometric changes. (# $p<0.05$ vs. baseline and * $p<0.05$ vs. saline)

FIGURE 4.

Contractile protein (actin, myosin, and troponin-I, C, and T) abundances were determined within remote, border, and infarcted regions of the LV. A region-specific decrease in the abundance of contractile proteins progressing from the remote region towards the infarct region was observed in both MI groups. CASPI was associated with a reduction in total contractile protein abundance compared to the saline group. However, lower molecular weight sub-fractions of troponin-T appeared decreased with CASPI suggesting less contractile protein degradation occurred. (#p<0.05 vs Control, *p<0.05 vs Saline, +p<0.05 vs Remote, ¶p<0.05 vs Border)

FIGURE 5.

Values for plasma-Pro-MMP-2 presented as a change from baseline. At 30 min post-reperfusion, plasma-Pro-MMP-2 was similarly decreased in both MI groups compared to baseline values suggesting that a concomitant increase in the active form of this MMP species had occurred. At 24 hrs and 7 days post-reperfusion, plasma-Pro-MMP-2 levels appeared to return to baseline values in the saline group. In contrast, at 24 hrs post-reperfusion, a shift was observed in the CASPI group. Specifically, plasma-Pro-MMP-2 was increased compared to baseline and saline values suggesting that a relative decrease in the abundance of active-MMP-2 had occurred. (# $p < 0.05$ vs Baseline and + $p < 0.05$ vs Saline)

FIGURE 6.

Caspase-3 activity within remote tissue collected at 7 days post-MI. Cell lysates obtained from control and infarcted pigs were separated into 2 samples/pig. A total of 1 μ L of 1mM Ac-DEVD-CHO inhibitor was added to 1 sample from each pig. Caspase-3 assay reactions were performed at room temperature on each sample and fluorescence was assessed in a microplate reader (excitation/emission: 485/535 nm). Similar degrees of protease activity were observed in the control and saline groups. In contrast, protease activity was significantly reduced in the CASPI group compared to control and saline values. The addition of the Ac-DEVD-CHO inhibitor attenuated caspase-3 activity in each group confirming that signal detection correlated with protease activity. (# p <0.05 vs Control, * p <0.05 vs Saline, + p <0.05 vs No Inhibitor)

FIGURE 7.

This *in-vitro* study examined the total amount of MMP proteolytic activity that would be affected by caspases. Homogenized LV tissue from control pigs (20 µg), purified fluorogenic substrate for MMP-1, 2, 3, 7, and 9 (Calbiochem), and activated caspase-3 (50 ng/mL, BD Biosciences) were prepared and combined with and without z-VAD (z-Val-Ala-Asp-fluoromethyl-ketone, 250 µM, Sigma) to assess the effects of caspase-3 upon *in vitro* MMP activity. Values for MMP-1, 2, 3, 7, and 9 are presented as a percentage of control (LV tissue and purified fluorogenic substrate, alone). MMP-1, 2, 3, 7, and 9 activities increased in the presence of caspase-3 compared to control values. In contrast, administration of the caspase inhibitor, z-VAD, abrogated the increase in MMP activity induced by activated caspase-3.

FIGURE 8.

The effects of increasing concentrations of activated caspases 1–10 (BioVision K233-10-25) on uniform concentrations of recombinant human Pro-MMP-2 (R&D Systems, 902-MP) were assessed in a purified *in-vitro* system devoid of other proteases and inhibitors which may be present in LV myocardial homogenates. MMP-2 and –9 standards (Chemicon, CC073) were used for molecular weight comparison. The zymogram demonstrates that activated caspases cleave the pro-form of MMP-2. Moreover, increasing concentrations of activated caspases are associated with further degradation of the initial MMP-2 cleavage product. Integrated optical densities were assessed for quantification.

Table 1Systemic Hemodynamics, Myocardial Injury, Myocardial Morphometrics, Regional Stress: **Effects of CASPI**

		MI	
	Control	Saline	CASPI
<u>Systemic Hemodynamics</u>			
Heart Rate (bpm)	93±4	95±3	104±7
Cardiac Output (L/min)	3.5±0.2	3.5±0.2	3.4±0.3
Mean Aortic Pressure (mmHg)	79±3	73±5	62±3 ^{#+}
LV Peak Systolic Pressure (mmHg)	101±3	91±6	81±3 [#]
LV End-Diastolic Pressure (mmHg)	5±1	11±2 [#]	10±1 [#]
Mean Pulmonary Artery Pressure (mmHg)	13±1	11±2	15±2
<u>Myocardial Injury</u>			
Infarct Size at 7 days (%)	-	51±8	42±8
Plasma Troponin-I at 24 hrs (ng/mL)	-	189±20	133±15 ⁺
Plasma Troponin-I at 72 hrs (ng/mL)	-	29±8	26±4
<u>Isolated Myocyte Dimensions</u>			
Length (μm)	136±4	141±4	128±3 ⁺
Width (μm)	45±3	33±2 [#]	37±1 [#]
<u>Collagen Biochemistry</u>			
Remote Region (mg/gdwt)	32±4	35±3	36±4
Border Region (mg/gdwt)	-	62±4 ^{#*}	57±7 ^{#*}
Infarct Region (mg/gdwt)	-	82±6 ^{#*¶}	86±8 ^{#*¶}
<u>Regional Radial Stress</u>			
Septal Wall (g/cm ²)	-	376±18	324±26
Posterolateral Wall (g/cm ²)	-	409±28	306±26 ⁺
Sample Size (n)	11	12	10

CASPI: IDN6734 (6 mg/kg bolus followed by 6 mg/kg/hr infusion for 24 hrs), a broad-spectrum caspase inhibitor, was instituted following 60 min of regional ischemia at the time of myocardial reperfusion.

[#] p<0.05 vs. Control

⁺ p<0.05 vs. Saline

^{*} p<0.05 vs. Remote

[¶] p<0.05 vs. Border