Pharmacologic inhibition of intracellular caspases after myocardial infarction attenuates left ventricular remodeling: A potentially novel pathway

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Objective: Myocyte death occurs by necrosis and caspase-mediated apoptosis in the setting of myocardial infarction. In vitro studies suggest that caspase activation within myocytes causes contractile protein degradation without inducing cell death. Thus, caspase activation may evoke left ventricular remodeling through 2 independent processes post-myocardial infarction. However, the effects of caspase activation on left ventricular geometry post-myocardial infarction remain unclear. This project applied broad-spectrum caspase inhibition to a chronic porcine model of myocardial infarction.

Methods: Coronary snares and sonomicrometry crystals in remote and area-at-risk regions were placed in pigs (n = 22, 34 kg). Geometric measurements at end diastole and end systole, including left ventricular area by echocardiography and interregional distance by sonomicrometry, were obtained at baseline. Coronary occlusion was instituted for 60 minutes, followed by reperfusion and repeated geometric measurements at 7 days, including left ventriculography. At reperfusion, pigs were randomized to saline (n = 12) or caspase inhibition (n = 10, IDN6734, 2 mg/kg intravenously, then 2 mg · kg · h for 24 hours) at a dose that achieved desired plasma concentrations (790 ± 142 ng/mL) as predicted by prior pharmacokinetic studies.

Results: Infarct size and 24-hour troponin-I values were not significantly different between the saline and caspase inhibition groups (51% ± 8% vs 42% ± 6% and 189 ± 20 ng/mL vs 152 ± 26 ng/mL, respectively, $P > .10$). At 7 days, end-diastole volume was increased in both groups compared with reference control values (47 ± 1 mL, $P < .05$), but it was decreased with caspase inhibition (72 ± 4 mL) compared with saline (84 ± 4 mL, $P < .05$). Similarly, end-diastole and end-systole areas increased by 32% ± 3% and 81% ± 16% in the saline group but were attenuated with caspase inhibition (19% ± 3% and 31% ± 10%, respectively, $P < .05$). End-diastole interregional distance increased by 30% ± 7% in the saline group but was attenuated with caspase inhibition (12% ± 5%, $P < .05$).

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Conclusion: Despite equivalent degrees of myocardial injury, caspase inhibition reduced post-myocardial infarction left ventricular remodeling as evidenced by multiple, independent assessments of left ventricular dilation. Thus, caspase activation alters left ventricular geometry in the absence of significant effects on myocardial injury.

Left ventricular (LV) remodeling is a structural consequence of prolonged myocardial ischemia or myocardial infarction (MI).[1] The extent of LV remodeling is commonly quantified by assessing changes in the geometry of the LV chamber (volume and dimension) that ensue after the onset of alterations in both the cellular and extracellular myocardial compartments. A cellular event that forms the underpinning of LV remodeling processes is ischemia-induced alterations in myocyte structure and viability.[2] Although MI has been clearly demonstrated to occur as a direct result of ischemia-induced myocyte necrosis, "programmed cell death," or apoptosis, has recently been described as an important contributing entity in the evolution of MI.[3,4] Moreover, recent in vitro and in vivo studies have demonstrated that modulation of the apoptotic cascade may improve myocardial viability[5-8] and function[5,8] in the context of short durations of ischemia-reperfusion (I/R). However, whether and to what degree the results of these past basic studies can be translated to large animal models of MI that may more closely resemble the clinical setting of MI remain unknown.

The caspases are an endogenous family of intracellular cysteine proteases that participate in critical steps of the apoptotic cascade in numerous pathologic processes including cerebrovascular accidents, neurodegenerative disorders, and acquired immunodeficiency syndrome.[9] More recently, caspases have been described to modulate myocyte apoptosis, particularly in the setting of myocardial ischemia.[10,11] Specifically, interruption of caspase activity using pharmacologic inhibition has been demonstrated to attenuate myocardial injury in vitro[12] and in vivo[8] after a limited period of regional ischemia. However, whether and to what degree caspase inhibition (CASPI) may affect LV geometry in the post-MI setting remain unknown. Accordingly, the present study examined the effects of broad-spectrum pharmacologic CASPI on regional and global LV geometry in a porcine model of MI.

Methods

Study Rationale and Overview

The present study used a chronic porcine model in which the effects of broad-spectrum pharmacologic CASPI on indices of LV remodeling were assessed after the induction of MI. Pigs were used as an animal model because they exhibit coronary artery anatomy and cardiac physiology similar to that of humans.[13] A broad-spectrum caspase inhibitor (IDN6734, Idun Pharmaceuticals, San Diego, Calif) was instituted immediately before myocardial reperfusion and was continued for a period of 24 hours post-MI. IDN6734 was administered at the time of reperfusion to simulate the common clinical scenario of MI in which pharmacologic intervention is administered, and coronary reperfusion is frequently reestablished only after a significant duration of ischemia. A broad-spectrum CASPI with a similar structure has been characterized and demonstrated to prevent caspase-induced cardiomyopathy.[14] 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%/1.5 L/min) and nitrous oxide (0.5 L/min). Through a left anterolateral thoracotomy, catheters (7-Fr) connected to subcutaneous access ports were sutured into the descending thoracic aorta and pulmonary artery. A disengaged coronary snare device was placed around the circumflex coronary artery immediately distal to the first obtuse marginal branch (Figure 1). Two pairs of piezoelectric crystals (2 mm, Sonometrics, Ontario, Canada) were advanced into the LV chamber and secured adjacent to the endocardial surface. The first crystal pair was placed between the first and second diagonal branches of the left anterior descending coronary artery, and the second pair was placed between the third and fourth obtuse marginal branches of the circumflex coronary artery. The distal ends of the sonomicrometry crystal cables coalesced into a single connector hub that was tunneled out of the chest and buried within a subcutaneous pocket along with the distal end of the coronary snare device. The incisions were closed in layers, and the pigs were allowed to recover.

Randomization. After 5 days of recovery, the pigs were randomized (Figure 1) in a blinded fashion to saline (n = 12) or CASPI (n = 10, IDN6734, 2 mg/kg intravenously [IV], followed by 2 mg · kg · h for 24 hours). The dosing regimen for IDN6734 achieved desired plasma concentrations (790 ± 142 ng/mL) as predicted by prior pharmacokinetic studies. A total of 11 normal, age-matched, noninstrumented pigs were included for comparison. Treatment codes were not broken until the entire study was completed.

Baseline measurements. Two-dimensional and M-mode LV echocardiographic images (ATL Ultramark VI, 2.25 MHz transducer, Bothell, Wash) were obtained from a right parasternal approach. The short and long-axis 2-dimensional targeted images

were gated to continuous electrocardiograms to define end systole and end diastole, and short- and long-axis LV areas were determined. The subcutaneous aortic and pulmonary artery ports were accessed to collect plasma, transduce arterial pressure, and deliver appropriate antiarrhythmics and heparin (150 U/kg, Pharmacia and Upjohn, Kalamazoo, Mich). The disengaged snare devices and sonomicrometry crystal hubs were exteriorized from their subcutaneous pockets in a sterile fashion. The sonomicrometry crystals were used to assess the distance between the remote and area-at-risk regions at end systole and end diastole.

MI induction. Coronary snare engagement resulted in proximal occlusion of the circumflex coronary artery and produced myocardial injury that was immediately confirmed by standard limb-lead electrocardiographic and hemodynamic changes. Episodes of ventricular tachycardia and fibrillation were terminated with lidocaine (50 mg, Elkins-Sinn, Cherry Hill, NJ) and external defibrillation, respectively. After 60 minutes of coronary occlusion, the snares were disengaged and the pigs were reperfused. Pigs randomized to CASPI received a bolus of IDN6734 (2 mg/kg, IV) immediately before reperfusion followed by a continuous infusion (2 mg/kg/h, IV) for a period of 24 hours. IDN6734 was administered through the pulmonary artery catheter. The disengaged snares and crystals hubs were returned to their subcutaneous pockets, and the wounds were closed.

Terminal measurements. At 7 days post-MI, echocardiographic and sonomicrometry crystal measurements were repeated. Subsequently, a full anesthetic induction and instrumentation procedure was performed as previously described to obtain systemic hemodynamic measurements, LV and pulmonary artery pressures, and indices of global function.[15] LV volumes were computed from ventriculograms as described previously.[16] The LV was explanted, and mid-ventricular, short-axis, and circumferential sections were obtained for tetrazolium chloride staining and infarct size estimation.[17] Infarct size was computed as a percentage of the myocardial area-at-risk. Systemic hemodynamics and indices of global function were similarly determined in the control pigs.

Data analysis. Changes in hemodynamic, functional, and geometric values between the saline and CASPI groups were analyzed with a 2-way analysis of variance followed by mean separation with pair-wise Bonferroni corrections. Values are presented as the mean and SEM.

Results

External defibrillation for ventricular fibrillation was required in 18% of the pigs during the 60-minute ischemic interval with no difference between MI groups (Pearson corrected χ^2 analysis, $P > .97$). There were no episodes of ventricular fibrillation during the reperfusion interval. At 24 hours post-MI, plasma cardiac troponin-I values were not significantly different between the saline and CASPI groups (189 ± 20 ng/mL vs 152 ± 26 ng/mL, respectively, $P = .21$) (Figure 2). At 7 days after coronary occlusion and release, myocardial infarct size was not statistically different between the saline and CASPI groups ($51\% \pm 8\%$ vs $42\% \pm 6\%$, respectively, $P = .40$) (Figure 2).

Steady-State Hemodynamics

Steady-state hemodynamics obtained at 7 days post-MI are presented in Table 1. LV end-diastolic pressure was increased in both MI groups compared with control values. There were no significant differences in heart rate, cardiac output, or LV-developed pressure between the control or MI groups. However, compared with control values, mean aortic and LV peak pressures were decreased in the CASPI group.

Interregional Geometry and Contractile Function

The distances, or chord lengths, between sonomicrometry crystals positioned within the remote and area-at-risk regions were determined at end diastole and end systole in both MI groups (Figure 3). The end-diastolic and end-systolic chord lengths were increased at 7 days post-MI in both MI groups compared with baseline values. However, end-diastolic and end-systolic chord lengths were reduced in the CASPI group compared with the saline group values. LV interregional shortening was determined at 7 days post-MI between the remote and area-at-risk regions in both groups as well (Figure 3). Interregional shortening was reduced in the saline group compared with baseline values. However, administration of CASPI preserved LV interregional shortening compared with saline group values.

Global Left Ventricular Geometry

Global LV geometry was assessed using long-axis echocardiography at baseline and at 7 days post-MI (Figure 4). LV end-diastolic and end-systolic areas were increased at 7 days post-MI in both groups compared with baseline values, but this was significantly attenuated with the institution of CASPI at the time or myocardial reperfusion compared with saline values (Figure 4). Left ventriculograms performed during terminal investigations provided additional independent assessments of global LV geometry and function (Table 1). Compared with control values, LV end-diastolic and end-systolic volumes were increased, and LV ejection fraction was decreased, in the saline group at 7 days post-MI. Administration of CASPI significantly attenuated LV dilation after MI and seemed to improve ejection fraction compared with saline values, although statistical significance was not reached.

Discussion

MI disrupts the dynamic architecture of the LV wall and can result in chamber dilation. This process, termed "LV remodeling," has been demonstrated to be an independent determinant of morbidity and mortality after MI.[18] Notably, pharmacologic strategies designed to interrupt specific cellular and extracellular pathways causative to post-MI LV remodeling, such as excessive neurohormonal stimulation, have had favorable effects on morbidity and mortality.[19]

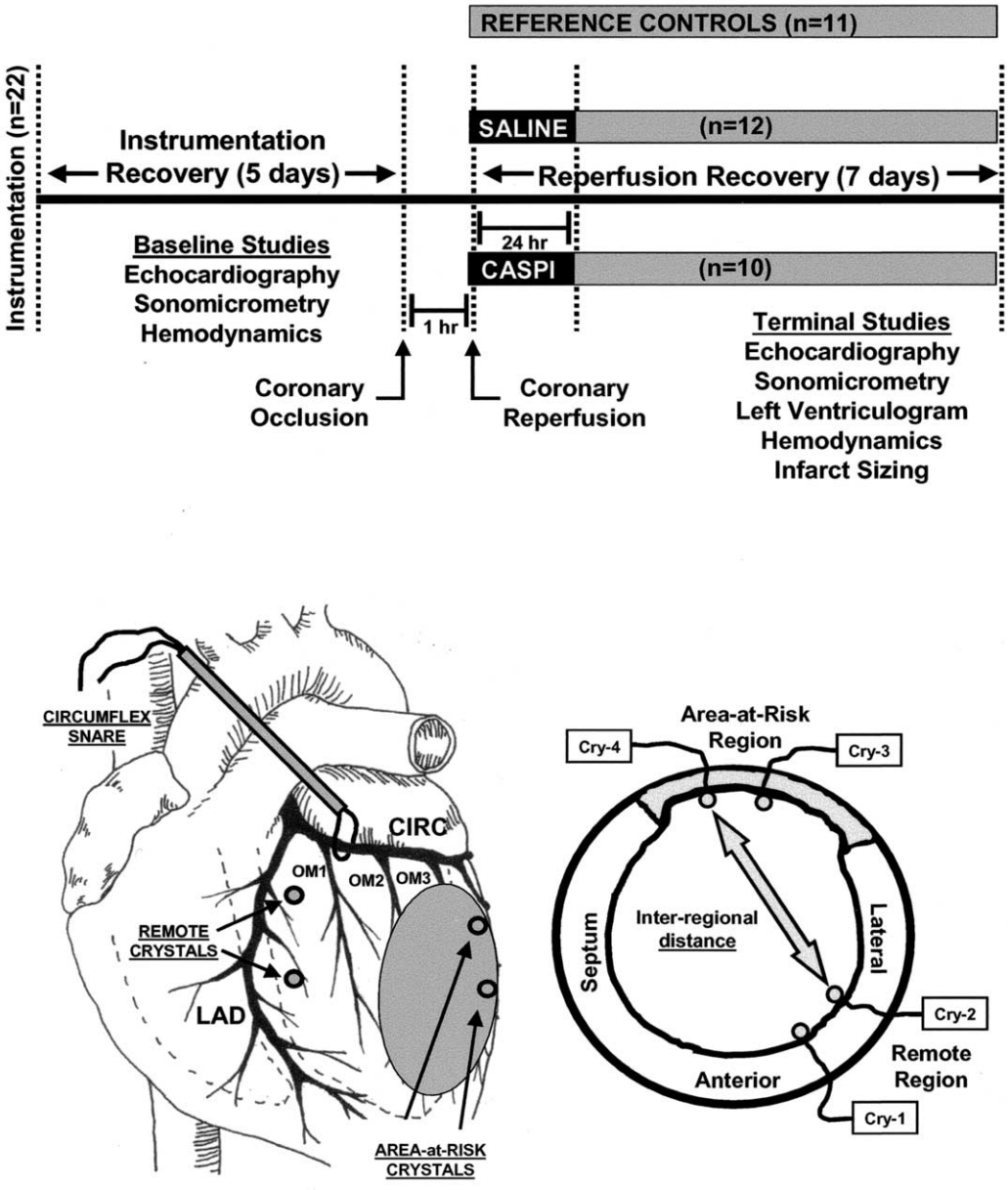

Figure 1. Top, The experimental design included a 5-day recovery period after basic instrumentation. After 60 minutes of regional myocardial ischemia, pigs were randomized to saline or CASPI groups. Terminal measurements obtained at 7 days post-MI included left ventriculograms. Bottom, left, A schematic of the heart after 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). Bottom, 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. *CASPI,* Caspase inhibition; *LAD,* left anterior descending.

Recent investigations suggest that modulation of intracellular caspases, the primary effectors of cell death by apoptosis, may hold significant therapeutic potential as a treatment modality in the setting of myocardial I/R.[5-8,12,20] Specifically, ischemia-induced myocardial injury was attenuated in vitro by interrupting intracellular caspase activity and by protecting against activated caspases through the use of pharmacologic[12] and genetic interventions,[5-7] respectively.

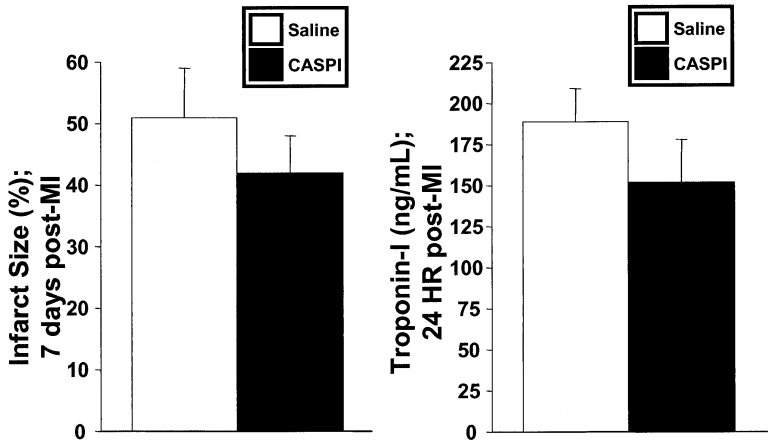

Figure 2. Myocardial injury was assessed by determining LV infarct size at 7 days post-MI (left) and plasma troponin-I values 24 hours after MI creation (right). There were no statistically significant differences between the MI groups with respect to these parameters. *MI,* Myocardial infarction.

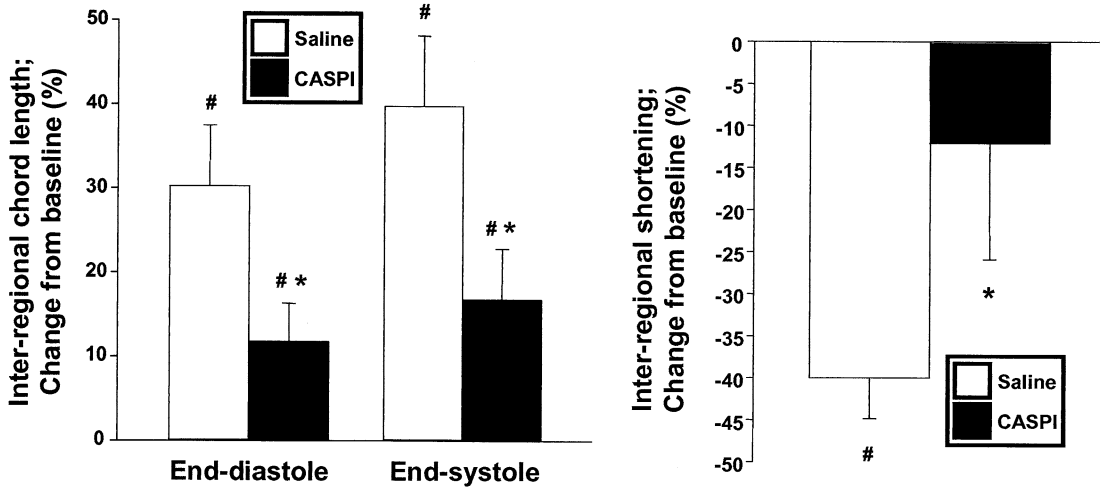

Figure 3. At baseline and at 7 days post-MI, end-diastolic and end-systolic chord lengths were determined between the remote and area-at-risk regions (left) yielding distances that approximated the diameter of the LV and facilitated assessments of interregional shortening (right). LV end-diastolic and end-systolic chord lengths were increased, and interregional shortening was reduced in the saline group at 7 days post-MI. CASPI significantly attenuated these geometric and functional changes. (#$P < .05$ vs baseline and *$P < .05$ vs saline.)

However, most of these past studies used ischemic intervals of short duration and examined myocardial injury, as assessed by infarct size as a primary end point. Thus, the effects of modulating caspase activity on LV remodeling in a large animal model of MI that may resemble a common clinical scenario remain to be defined. Accordingly, the primary objective of the present study was to test the central hypothesis that broad-spectrum CASPI would favorably alter regional and global LV remodeling in a chronic porcine model of MI. The unique findings of the present study were 2-fold. First, when instituted at the time of reperfusion, broad-spectrum CASPI attenuated regional and global LV remodeling during the acute post-MI period. Second, the observed reductions in LV dilation post-MI were achieved in the absence of significant effects on absolute myocyte troponin-I release and MI size.

Caspases and the Apoptotic Cascade

Prolonged periods of myocardial ischemia evoke myocyte death through 2 distinct and time-dependent processes, namely, necrosis and apoptosis.[10,21] In past experimental settings, most myocytes that succumbed to acute and per-

TABLE 1. Systemic hemodynamics, left ventricular geometry, and myocardial injury 7 days post-myocardial infarction: Effects of caspase inhibition

	Control	Saline	CASPI
Systemic hemodynamics			
Heart rate (beats/min)	93 ± 4	95 ± 3	96 ± 5
Cardiac output (L/min)	3.5 ± 0.2	3.5 ± 0.2	3.3 ± 0.3
Mean aortic pressure (mm Hg)	79 ± 3	73 ± 5	67 ± 4*
LV peak systolic pressure (mm Hg)	101 ± 3	91 ± 6	86 ± 5*
LV end-diastolic pressure (mm Hg)	5 ± 1	11 ± 2*	14 ± 1*
Mean pulmonary artery pressure (mm Hg)	13 ± 1	11 ± 2	14 ± 2
LV volumes			
End-diastolic volume (mL)	47 ± 1	84 ± 4*	72 ± 4*†
End-systolic volume (mL)	12 ± 2	47 ± 4*	38 ± 4*
Ejection fraction (%)	76 ± 4	45 ± 4*	49 ± 4*
Myocardial injury			
Infarct size (%)	—	51 ± 8	42 ± 6
24-h cardiac troponin-I (ng/mL)	—	189 ± 20	152 ± 26
Sample size (n)	11	12	10

CASPI: IDN6734 (2 mg/kg bolus followed by 2 mg · kg · h infusion for 24 h), a broad-spectrum caspase inhibitor, was instituted after 60 min of regional ischemia at the time of myocardial reperfusion.
MI, Myocardial infarction; *CASPI,* caspase inhibition; *LV,* left ventricular.
*$P < .05$ vs control.
†$P < .05$ vs saline.

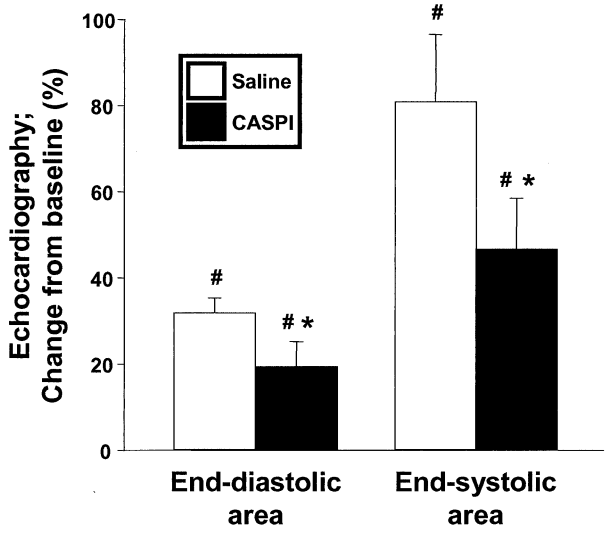

Figure 4. LV end-diastolic and end-systolic areas presented as changes from baseline. At 7 days post-MI, LV areas were increased in both MI groups compared with baseline values. However, institution of CASPI conferred significant reductions in LV dilation compared with saline values. (#$P < .05$ vs baseline and *$P < .05$ vs saline.)

manent myocardial ischemia were believed to have died by necrosis.[22] On the contrary, recent studies have demonstrated that apoptosis, an energy-dependent process, represents a particularly important myocyte death mechanism in the setting of I/R.[5-8,12,20] Through the use of genetic manipulation and pharmacologic CASPI, these past investigations provided proof of concept that caspases are intricately involved in the apoptotic process. However, as evidenced by a recent in vitro investigation,[23] activation of intracellular caspases within isolated myocytes was demonstrated to evoke deteriorations in contractile function through the degradation of cytosolic contractile elements without a cellular commitment to apoptosis. Taken together, the results of these basic investigations indicate that intracellular caspases likely play multiple roles within myocytes. The present study builds on the results of these past investigations by using broad-spectrum CASPI in a chronic large animal model of MI. This unique model not only facilitated assessment of myocardial injury during the acute and subacute peri-infarction periods but also allowed subsequent changes in regional and global LV geometry post-MI to be assessed.

Caspase Inhibition and Myocardial Injury

Numerous past studies have demonstrated that modulation of caspase activity attenuated myocardial injury in the setting of I/R.[5-8,12,20] For instance, Gustafsson and investigators[7] used transgenetic rodent models to report that manipulation of caspase activity resulted in significant reductions in infarct size and creatine kinase efflux. However, many of these preclinical investigations used limited durations of myocardial ischemia in conjunction with isolated whole-heart preparations.[5-7,12] The present study is unique in that a chronic large animal model of MI was used that more closely recapitulated the clinical context. Because the targeted myocardial regions used in the present study were devoid of significant collateral vessels,[13] an extensive amount of tissue loss was caused by the prolonged ischemic interval as evidenced by the marked elevations in plasma cardiac troponin-I values observed in both MI groups at 24 hours post-MI. Moreover, the substantial plasma release of troponin-I at 24 hours may indicate that necrosis represented the dominant pattern of cell death during the acute peri-infarction period. However, whether and to what degree myocyte apoptosis contributed to the increase in plasma troponin-I remain unknown. Institution of broad-spectrum CASPI immediately before reperfusion, although unable to salvage myocardial tissue lost by necrosis during the ischemic interval, may have attenuated additional myocyte death within the infarct border zones by apoptotic cascades. Preservation of myocyte viability within the relatively small mass of the infarct border zone may provide an explanation as to why CASPI seemed to reduce infarct size and troponin-I release without reaching statistical signifi-

cance. Histologic examination of border-zone tissue may be warranted to address this issue more closely. Nevertheless, the results of the present study clearly indicate that broad-spectrum CASPI attenuated LV remodeling post-MI in the absence of significant effects on myocyte troponin-I release and MI size.

Left Ventricular Remodeling After Myocardial Infarction

The potential implications of apoptosis in the pathogenesis of LV remodeling post-MI have been studied.[24,25] For example, Palojoki and investigators[25] used terminal deoxynucleotide transferase-mediated dUTP nick-end labeling (TUNEL) methodology in a chronic rodent model of MI to demonstrate that apoptosis occurred within both remote and border-zone regions. In the present study, the effects of CASPI on regional and global LV geometry were evaluated at 7 days post-MI. Because myocardial apoptosis peaks approximately 12 hours post-MI,[26] qualitative and quantitative assessments of myocardial apoptosis were not used. Therefore, whether and to what degree CASPI attenuated apoptosis within the infarct border zones remain to be determined. Use of TUNEL, fluorescent-annexin V staining, or caspase-3 assays would likely yield important information with respect to this matter. Nevertheless, in the present study, broad-spectrum CASPI was associated with significant reductions in multiple, independent assessments of LV remodeling post-MI, namely, LV area (echocardiography), volume (ventriculography), and interregional chord length (sonomicrometry). It is probable that broad-spectrum pharmacologic inhibition attenuated the effects of caspase activation within the border zone evoked by the generation of reactive oxygen species on reperfusion and increased LV wall stress post-MI, factors known to induce apoptosis in vitro.[27,28] Institution of CASPI at the time of reperfusion, and throughout the following 24 hours, likely rendered the caspases incapable of executing not only the apoptotic cascade but also the cleavage of other intracellular substrates. CASPI may have attenuated degradation of contractile proteins located within viable myocytes contained in the infarct border zone, thus providing an explanation for the improved interregional function and trend toward increased ejection fraction associated with the CASPI group. Subsequent attempts to quantify the degree of myocardial contractile protein degradation that occurred within each MI group may therefore prove useful.

Study Limitations and Clinical Implications

The present study used broad-spectrum CASPI in a chronic large animal model to assess changes in LV geometry post-MI. Therefore, whether and to what degree CASPI attenuated apoptosis within the infarct border zones remain to be determined. Moreover, whether and to what degree CASPI modulates LV geometry in the setting of permanent coronary artery occlusion remain to be addressed. Nevertheless, the results of the present study indicate that caspases may play multiple roles in the structural dynamics of the myocardium post-MI. Broad-spectrum CASPI instituted at the time of reperfusion attenuated regional and global LV remodeling post-MI. Thus, strategies that modulate caspase activation may hold significant therapeutic potential in the setting of MI.

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Discussion

Dr Y. Joseph Woo *(Philadelphia, Pa).* Could you perhaps elaborate a little more about exactly what aspect of your apoptotic cascade that this agent blocks?

Second, did you measure your regional changes in contractility from within the infarct to outside, effectively across the border zone where most of this antiapoptotic effect would have its greatest benefit?

Last, your laboratory probably has the most expertise in the world regarding extracellular matrix modification, and I was wondering if you could comment a little bit more on potential relationships there.

Dr Yarbrough. With regard to your first question, we use a broad-spectrum CASPI, and essentially it inhibited caspases 3, 6, 8, and 9, the major players in the apoptotic cascade. This was determined essentially by in vitro investigations in which you could take pure caspases, substrates for those caspases, and then add an inhibitor and determine the IC50 and essentially try to achieve that same compound concentration in vivo, which is what we accomplished with this study.

The crystals were placed such that the border zone was included. So the 2 crystals in the remote region were placed in the left anterior descending region, and then the 2 crystals in the area-at-risk region were placed on the posterior wall. So we did include the border zone with respect to that interregional assessment. It essentially approximated a diameter of the left ventricle, kind of giving us a pseudo-global assessment of function.

With regard to your final question, the results of this study essentially mirror what we have seen with broad-spectrum matrix metalloproteinase inhibition in the MI model; that is, we see beneficial attenuations in LV remodeling with not much of a functional effect, at least within 1 or 2 weeks of the onset of drug administration. In addition to that, we know that matrix metalloproteinases are activated, not only more abundant, but more activated in the myocardial interstitium within minutes of ischemia. So taken together, we believe that somehow or another administration of this agent is affecting matrix metalloproteinase activity and that this is translating into beneficial attenuations in LV remodeling.

Dr Syed M. Quadri *(Toronto, Ontario, Canada).* I am wondering if you measured caspase activity in the tissue, in the myocardium specifically, to verify that this was the pathway involved?

Dr Yarbrough. We are in the process of doing that now. A lot of the studies that we have looked at previously used ischemic intervals of shorter duration, and they harvested tissue within hours of the I/R injury. This study was a chronic study. We went 7 days. So short of actually performing an endocardial biopsy during the course of the study, we really only had tissue available at 7 days post-MI.

We are in the process of performing the caspase 3 activity assay in the remote region to try to assess whether or not there was any difference between groups. I suspect that these data are going to show very small differences. I think the bigger picture will be to look at matrix metalloproteinase abundances and activities in those regions at the same time point, and I think we will see a difference with respect to that parameter.