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In vivo gene delivery of XIAP protects against myocardial apoptosis and infarction following ischemia/reperfusion in conscious rabbits

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Abstract

Aims—We tested the hypothesis that an *in vivo* gene delivery of the pro-survival protein XIAP (X-chromosome linked inhibitor of apoptosis protein) protects against myocardial apoptosis and infarction following ischemia/reperfusion.

Main Methods—Nineteen rabbits were chronically instrumented with an hydraulic occluder placed around the circumflex coronary artery. Adenovirus harboring XIAP (Ad.XIAP; 1×10^{10} pfu/ml) or β -galactosidase (5×10^9 pfu/ml), as a control, was constructed and transfected into the heart using a catheter placed into the left ventricle accompanied by cross-clamping. 1-2 weeks after gene delivery, myocardial ischemia was induced by a 30-min occlusion followed by reperfusion for four days. Protein expression was determined by Western blot and Apoptosis (% of myocytes) was quantified by TUNEL staining.

Key Findings—Myocardial infarct size, expressed as a fraction of the area at risk, was reduced in Ad.XIAP (n=5) compared to control (n=7) rabbits ($21 \pm 3\%$ vs. $30 \pm 2\%$, $p < 0.05$). Apoptosis was reduced in Ad.XIAP rabbits compared to control rabbits ($2.96 \pm 0.68\%$ vs. $8.98 \pm 1.84\%$, $p < 0.01$). This was associated with an approximate 60% decrease in the cleaved caspase-3 level in Ad.XIAP rabbits compared to control rabbits.

Significances—The current findings demonstrate that overexpression of XIAP via *in vivo* delivery in an adenovirus can reduce both myocardial apoptosis and infarction following ischemia/reperfusion, at least in part, due to the ability of XIAP to inhibit caspase-3. These findings confirm previous work suggesting a link between myocardial apoptosis and infarction i.e., anti-apoptotic therapy was effective in reducing myocardial infarct size.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

Keywords

heart; ischemia; apoptosis; XIAP; gene therapy

Introduction

Caspases are a family of cysteine proteases, which are central regulators of apoptosis. The binding of pro-apoptotic ligands to their receptors initiates a caspase cascade, which results in the initiation and execution of apoptosis (McConkey 1998). The caspase cascade consists of activation of initiator caspases (caspases 8, 9, 10, and 12), which cleave and activate downstream effector caspases (caspases 3 and 7). These latter caspases, in turn, cleave nuclear proteins, thus inducing apoptosis (Boatright and Salvesen 2003; Denault and Salvesen 2002; Stennicke, et al. 2002). Activation of caspases can also occur via direct stimulation of a receptor-independent pathway involving mitochondrial dysfunction and release of cytochrome c (Boatright and Salvesen 2003; Denault and Salvesen 2002; Stennicke, et al. 2002).

In a previous study, we demonstrated, both in a swine model and in humans, a novel mechanism by which brief periods of ischemia cause activation of an endogenous gene program of cell survival, thus explaining the sustained viability of the hibernating myocardium (Depre, et al. 2004). The gene program includes the genes and corresponding proteins that are known to protect the heart by blocking apoptosis and promoting cytoprotection and cell growth. Among these clusters of genes is the inhibitor of apoptotic proteins (IAP), which exerts its effect by binding and inhibiting the action of the caspases (Deveraux, et al. 1998; Riedl, et al. 2001).

The current study was designed to test the hypothesis that overexpression of a member of the IAP family, XIAP (X-chromosome linked XIAP), can reduce apoptosis and infarct size following ischemia/reperfusion. Overexpression of XIAP was accomplished *in vivo* in rabbit hearts by transduction of adenovirus harboring the XIAP gene (Ad.XIAP) (Yamamoto, et al. 2001). Myocardial ischemia/reperfusion was produced under the conscious condition to avoid the effect of background anesthesia and acute surgical stress. The effect of treatment with Ad.XIAP on apoptosis, infarct size, and caspase 3 levels was evaluated four days after reperfusion, at which time the area of necrosis was readily visible and included apoptotic cells.

Methods

The study was conducted after approval from the Institutional Animal Care Committee. Animals used in this study were maintained in accordance with the “*Guide for the Care and Use of Laboratory Animals*” (National Research Council, revised 1996).

Construction of Adenoviral Vectors and Gene Delivery

Replication deficient human adenovirus type 5 (devoid of E1 and E3) harboring XIAP (Ad.XIAP) and β -galactosidase (Ad. β -gal) genes, as a control, were constructed as previously described (Yamamoto, et al. 2001).

Nineteen rabbits (2-5 kg) were sedated with acepromazine (0.5 mg/kg, i.m.), ketamine (35 mg/kg, i.m.), and xylazine (5 mg/kg, i.m.). General anesthesia was maintained with isoflurane (0.5-2.0 vol. %). A left thoracotomy was performed via the fourth intercostal space, the pericardium was opened, and 5-0 suture was placed at the apex of the left ventricle. Adenovirus harboring the XIAP gene (1×10^{10} pfu/ml) or the β -gal gene (5×10^9

pfu/ml) was transfected into the heart using a catheter placed into the left ventricle accompanied by cross-clamping as described previously (Hajjar, et al. 1998). Briefly, in the cross clamping method, an 18 G catheter containing 1ml of adenovirus was advanced from the apex of the left ventricle to the aortic root. While the aorta and pulmonary arteries were clamped distal to the site of the catheter using a 1 silk suture, the 1 ml solution contained adenovirus was injected. The clamp was maintained for 45 sec so that the heart pumped against a closed system.

In XIAP group (n=11), the rabbits were transfected with adenovirus harboring the XIAP gene (1×10^{10} pfu/ml); six of these rabbits (not subjected to an ischemia/reperfusion protocol) were used in validation studies to verify that an increased myocardial expression of XIAP was maintained over the three week duration of the study. Five rabbits were used to assess the effect of Ad.XIAP injection on myocardial apoptosis and infarct size following ischemia/reperfusion, as described below. In Control group (n=8), four rabbits were received the β -gal gene (5×10^9 pfu/ml) and four rabbits received saline and served as a non-viral control. These eight rabbits were subjected to the same surgical procedures as described above for the XIAP group.

Following completion of the adenovirus injection, a hydraulic occluder was placed around the circumflex coronary artery to allow induction of myocardial ischemia and ECG wires were implanted under the skin for the measurement of heart rate. The area at risk was confined to approximately 30-40% of the LV in order to minimize the probability of ventricular fibrillation during ischemia/reperfusion. The chest was closed and the animal was allowed to recover.

One-two weeks after gene delivery when the level of XIAP protein expression had stabilized (>2 fold increase; Figure 1), while the rabbit was in the conscious condition, myocardial ischemia was induced by a 30 min coronary occlusion followed by reperfusion for four days. 10 min prior to the coronary occlusion, the rabbit was sedated with diazepam (4 mg/kg, i.v.). This ischemia/reperfusion protocol was based on previous work indicating that it was adequate to cause significant apoptosis and myocardial infarction in the conscious rabbit (Aouam, et al. 2005). No antiarrhythmic drugs were given during myocardial ischemia/reperfusion.

Determination of myocardial area at risk and infarction size

After four days of coronary reperfusion, at a time when myocardial infarctions could be clearly identified and delineated grossly (Fishbein, et al. 1981; Huang, et al. 1999), the rabbit was euthanized by an overdose of pentobarbital (>100 mg/kg, i.v.) and the heart was excised. The area at risk (AAR) was identified using a dual perfusion technique, i.e., the coronary artery supplying the ischemic region was ligated with a 4-0 suture while Alcian Blue dye was injected into the non-ischemic region as described previously (Huang, et al. 1999). After completion of perfusion, the heart was cut into four short-axis slices from apex to base and then fixed in 5% formalin for 1 day. Each slice was weighed and the basal surface imaged using a digitizing program (ImageJ). Images were traced to calculate both AAR, identified by the blue dye exclusion, and infarcted tissue from the area of grossly identified necrosis, readily visible following four days of reperfusion. Mallory trichrome staining was used to compare ischemia/reperfusion-induced tissue damage for the Ad.XIAP transfected and control hearts on a histological level. Samples from three hearts in each group were used in these assays.

TUNEL Assay

Samples of myocardium (Control, n=5; Ad.XIAP, n=4) were obtained from the ischemic (but non-infarcted) and non-ischemic regions of the left ventricle and used for assessment of apoptosis. Myocardial apoptosis was assessed from DNA fragmentation, as determined by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay (Yang, et al. 2001). Two sections from each hearts were used to count the number of apoptotic cells. Data were reported as the ratio of TUNEL-positive cardiomyocytes to the total number of cardiomyocytes in both zones. Tissue sections were also counterstained with rhodamine-conjugated wheat germ agglutinin (WGA) to differentiate between apoptosis in nonmyocytes and myocytes.

Western Blotting

Western blotting was used to evaluate myocardial expression of XIAP and cleaved caspase-3, as described previously (Morisco, et al. 2001). Samples of myocardium were obtained from the ischemic region of the left ventricle. These samples were homogenized in ice-cold lysis buffer (10mM Tris pH 7.5, 100 mM NaCl, 1 mM EDTA, 3 µl/ml protease inhibitors' cocktail from Sigma) with 2% SDS and stored at -80°C before analysis. Equal amounts of protein (50 µg) were solubilized in 3-fold volume of sample buffer (62.5 mM Tris-HCl, pH 6.8, 31.5% glycerol, 2% SDS, 0.01% bromophenol blue, 5% β-mercaptoethanol) in boiling water for 5 minutes. Protein samples were subsequently resolved on 7 or 10% SDS-PAGE gels (Biorad) using the Biorad Mini-gel system. Gels were transferred to PVDF membranes (Immobilon-P, Millipore) using a wet transfer apparatus (Biorad) with 20% methanol, 25 mM Tris pH 8.3, and 192 mM glycine buffer. The primary anti-cleaved caspase-3 (1:500 dilution; Cell Signalling) and anti-XIAP (1:1000; MBL) and anti-XIAP were used. Primary antibodies were detected using anti-mouse and anti-rabbit IgG conjugated to HRP (Biorad) and enhanced chemiluminescence (Amersham). All western blot exposures were in the linear range of detection, and the intensities of the resulting bands were quantified by densitometry (Corel Photo).

Statistical Analysis

Data are presented as mean ± SE. Differences between the Control and XIAP groups were evaluated using the Student's t test for unpaired samples. The effect of ischemia on heart rate within a group was evaluated using the Student's t test for paired samples. Differences were considered significant when p<0.05.

Results

Expression of XIAP protein in vitro and in vivo

Figure 1 demonstrates the expression of XIAP in myocardial samples obtained from hearts that were injected *in vivo* with Ad.XIAP. An enhanced expression of XIAP (>2 fold) was maintained for the 3 week period following gene delivery.

Effects of XIAP Gene Delivery on Myocardial Infarct Size

During the *in vivo* injection of the adenoviral solution (while the aorta and pulmonary artery were cross clamped), the right and left ventricles became visibly pale as clear viral solution was supplied to the myocardium via the coronary arteries. During the injection, heart rate decreased markedly but recovered to baseline within 30 s of clamp release.

Since findings were the same for the β-gal and virus-free rabbits, these data were combined and served as control responses. Heart rate prior to myocardial ischemia in Ad.XIAP and

control rabbits was similar (271 ± 15 and 258 ± 20 beats/min, respectively). Coronary occlusion did not have an effect on heart rate in either group.

Figure 2 summarizes the findings for myocardial infarct size and area at risk for the control and Ad.XIAP groups. Myocardial infarct size, expressed as a fraction of the area at risk, was reduced in Ad.XIAP hearts compared to control hearts (21 ± 3 vs. $30 \pm 2\%$, $p < 0.05$). The ratio of area at risk to the total left ventricle weight was similar for Ad.XIAP and control hearts (33 ± 3 vs. $34 \pm 6\%$, respectively). Figure 3 presents representative sections stained with Mallory's trichrome stain, demonstrating that viable myocytes were present within the infarcted region of the Ad.XIAP-transfected heart but not within the infarcted region of the control heart.

Effects of XIAP Gene Delivery on Cardiomyocyte Apoptosis

Figure 4A presents representative TUNEL measurements of apoptotic cells in control (a and c) and Ad.XIAP (b and d) hearts, demonstrating a reduction in apoptotic cells in the Ad.XIAP transfected myocardium. Use of counterstaining with WGA (upper panels) localized apoptosis within the cardiomyocytes. Figure 4B summarizes the apoptosis results. It shows that apoptosis was reduced by 67% in Ad.XIAP ($n=4$) hearts compared to control ($n=5$) hearts ($2.96 \pm 0.38\%$ vs. $8.98 \pm 1.84\%$, $p < 0.05$). Apoptosis was also reduced by 73% in the non-ischemic region of the Ad.XIAP hearts (XIAP: $0.04 \pm 0.01\%$ vs. Control: $0.15 \pm 0.03\%$, $p < 0.05$).

Protein Expression of XIAP and Cleaved Caspase-3

Figure 5A presents the protein expression of XIAP and cleaved caspase-3 in Ad.XIAP ($n=4$) and Control ($n=4$) hearts following four days of reperfusion. The data demonstrate a greater than 2-fold increase in expression of XIAP accompanied by an approximate 60% decrease in expression of cleaved caspase-3 in Ad.XIAP hearts compared to control hearts (Figure 5B).

Discussion

Cardioprotective effects of Ad.XIAP

Our main findings were 1) an *in vivo* gene delivery of the pro-survival protein XIAP, using an adenovirus vector, reduced myocardial infarct size following ischemia/reperfusion in conscious rabbits and 2) this cardioprotective effect was associated with decreases in apoptosis and expression of the pro-apoptotic protein, cleaved caspase-3.

The current findings using adenovirus harboring XIAP are consistent with a number of studies demonstrating that modulation of apoptotic pathways can reduce myocardial infarct size in animal models and in humans (Anversa, et al. 1998; Gottlieb, et al. 1994; Haunstetter and Izumo 2000; Kajstura, et al. 1996; Olivetti, et al. 1991; Saraste, et al. 1997). For example, blocking the release of cytochrome c by overexpression of anti-apoptosis genes, such as Bcl-2 and Bcl-xL, reduced both myocardial apoptosis and infarct size following ischemia/reperfusion (Chatterjee, et al. 2002; Huang, et al. 2003). Similar results were obtained using systemic administration of pharmacologic caspase inhibitors (Holly, et al. 1999; Huang, et al. 2003).

The mechanism by which the Ad.XIAP-induced decrease in myocardial apoptosis resulted in a reduction in myocardial infarct size is uncertain. Two potential mechanisms can be proposed. One mechanism is based on the assumption that myocyte apoptosis represents a major form of cell death in the infarcted region. Although the absolute decrease in the number of apoptotic cells caused by Ad.XIAP in the present study was modest, it should be kept in mind that, because of technical reasons, these measurements were obtained in the

non-infarcted myocardium within the ischemic region, but not within the infarct itself. Work in a rat model of myocardial ischemia/reperfusion has suggested that the number of apoptotic cells in infarcted myocardium was 6-times that of necrotic cells (Anversa, et al. 1998). If a similar ratio between apoptotic and necrotic cells was evident in the infarcted region of the rabbit hearts in the present study, it could explain how treatment with Ad.XIAP produced such a marked decrease in infarct size. Another potential mechanism is based on the proposition that apoptotic cells **become** necrotic cells during reperfusion, i.e., apoptosis-necrosis continuum, thus extending the size of the infarct (Zhao and Vinten-Johansen 2002), and that Ad.XIAP treatment reduced this effect. However, Zhao et al. (Zhao and Vinten-Johansen 2002) demonstrated in a canine model of myocardial ischemia/reperfusion that the development of apoptosis and necrosis have different time courses; the extent of necrosis peaks at 24 hr reperfusion and remains constant thereafter, whereas the appearance of apoptotic cells in the ischemic region progressively increases up to 72 hr of reperfusion. Thus, if this mechanism were operative, it would only be during early reperfusion.

The findings using Mallory's trichrome stain revealed the presence of numerous surviving myocytes within the infarct of the Ad.XIAP hearts but not within that of the control hearts (Figure 4). This is noteworthy since it implies that our measurements of myocardial infarct size actually underestimated the beneficial effect of overexpression of XIAP.

An issue warranting address is whether the Ad.XIAP delayed, rather than permanently inhibited, infarct development. With this possibility in mind, hearts were obtained for determination of myocardial infarct size after 96 hrs of reperfusion, which is four times the duration of reperfusion required for maximal infarct size development, as shown by Zhao et al (Zhao and Vinten-Johansen 2002). Although it is theoretically possible that infarct size would have been greater if we had allowed reperfusion to proceed for a longer duration, this seems highly unlikely.

Chua et al (Chua, et al. 2007) recently reported that overexpression of another member of the IAP family, IAP-2, in transgenic mice reduced apoptosis and protected against myocardial ischemia/reperfusion injury. But these findings were potentially confounded by the influence of compensatory changes in related proteins that are frequently observed in transgenic mice (Chu, et al. 1996; Giordano, et al. 1997; He, et al. 1997). Indeed, XIAP-deficient mice have been reported to upregulate the proteins c-IAP1 and c-IAP2, which likely explains why these transgenic mice showed no impairment to induction of caspase-dependent or caspase-independent apoptosis (Harlin, et al. 2001). Our use of catheter-based adenoviral gene transfer to directly express the gene for XIAP in the myocardium on a short-term basis minimized the possibility of other protein changes (Chu, et al. 1996; Giordano, et al. 1997; He, et al. 1997). Another advantage of our approach is that it has been used commonly in human cardiovascular gene therapy protocols, which increases the clinical applicability of our findings (Isner 2002).

Our findings indicated that a decrease in cleaved caspase-3 levels accompanied the decrease in apoptosis in XIAP treated hearts. This is consistent with the well known ability of XIAP to directly inhibit caspase 3, as well as caspase 7 (Deveraux, et al. 1997). Another potential anti-apoptotic mechanism in the present study was the ability of XIAP to prevent the proteolytic processing of pro-caspases 6 and 7 by blocking the cytochrome c-induced activation of pro-caspase 9 (Deveraux, et al. 1998). In addition to a direct inhibition to myocyte apoptosis, XIAP has been demonstrated to have effects in vascular endothelial cells, which could indirectly provide protection to myocytes subjected to ischemia/reperfusion. These effects include a modulation of endothelial apoptosis and cell signaling molecules, and an increase in NO production by releasing endothelial NO from caveolin-1 (Hofer-Warbinek, et al. 2000; Kim, et al. 2008; Stehlik, et al. 1998).

Critique of Methods

In the present study, the effectiveness of gene transfer was a critical issue. We initially confirmed this in *in vitro* studies in cos7 cells, which demonstrated a concentration-dependent expression of XIAP following treatment with Ad.XIAP (Data not shown). We adopted the technique of Hajjar et al. (Hajjar, et al. 1998) to administer Ad.XIAP *in vivo*. Adenovirus was injected into the left ventricular cavity while the aorta and pulmonary artery were clamped. The appearance of clear viral solution flowing through the superficial coronary arteries provided visible evidence that the entire ventricular surface was exposed to the virus. Our findings during ischemia/reperfusion indicated that XIAP expression was enhanced at the time that myocardial apoptosis and infarction were reduced (Figure 5A). Our validation studies demonstrated that XIAP expression did not diminish during for the 3 week period following gene delivery (Figure 1).

Endogenous XIAP, rather than another related protein, was used in the present study because 1) it is ubiquitous being expressed in all normal tissues from viruses to mammals, 2) it has been identified as the most potent caspase inhibitor, targeting caspases 3, 7, and 9, and 3) it is known to target the caspases directly rather than indirectly (Deveraux, et al. 1998; Riedl, et al. 2001; Roy, et al. 1997; Salvesen 2002). The rabbit was chosen for use in the present study because of cost (a larger animal would have required a much larger, prohibitively expensive, quantity of virus) and because the native collateral coronary circulation is sparse in the rabbit. Nevertheless, caution should be exercised in extrapolating the current findings to the clinical situation because of the much higher resting heart rates, and presumably a higher myocardial metabolic demand and greater vulnerability to ischemia in the rabbit compared to man.

Several additional limitations of the study warrant address. One limitation was the lack of hemodynamic monitoring. The small size of the animal, the long duration (approximately 2 weeks) between the time of surgery and the carrying out of the ischemia/reperfusion protocol resulting in a high probability for the formation of clots, and our desire to perform this protocol in the conscious state were factors that precluded the implantation of catheters for measurements of arterial blood pressure, left ventricular pressure, or LV dp/dt_{max} . ECG electrodes which were implanted chronically under the skin at the time of surgery provided measurements of heart rate during the ischemia/reperfusion protocol. These values were similar for the rabbits of all experimental groups. Since heart rate should vary inversely with arterial pressure via the baroreceptor reflex in the conscious state, the constancy of heart rate suggested no inter-group differences in arterial pressure, as well. Although echocardiographic measurements of global cardiac function, e.g., ejection fraction, were theoretically possible, it is doubtful that they would have revealed a salutary effect for the XIAP treatment because 1) the size of the area at risk was, by design, limited to only a portion (30-40%) of the LV, and 2) the magnitude of the infarct size decrease (from 30 to 21 % of the area at risk) was moderate in magnitude.

Another possible limitation was that spillover of the virus into the systemic circulation could have resulted in it being extracted by another organ, e.g., the brain, causing indirect effects on cardiac function and altering vulnerability of the myocardium to ischemia/reperfusion induced injury. However, due to dilution of the virus in the systemic blood, we would expect its concentration in any individual non-cardiac tissue to be minimal.

Finally, although TUNEL staining is the most common technique to quantify apoptosis in fixed tissues, it has been criticized because of an inability to discriminate apoptotic from necrotic cells (Kelly, et al. 2003). Mindful of this potential limitation, we obtained tissue exclusively from non-necrotic areas for our assessments of apoptosis by the TUNEL technique and supported these data with measurements of caspase-3 expression.

In summary, the current findings demonstrate that overexpression of XIAP via *in vivo* delivery in an adenovirus can reduce both myocardial apoptosis and infarction following ischemia/reperfusion, at least in part, due to the ability of XIAP to inhibit caspase-3. These findings confirm previous work suggesting a link between myocardial apoptosis and infarction i.e., anti-apoptotic therapy was effective in reducing myocardial infarct size (Chatterjee, et al. 2002; Huang, et al. 2003). They also provide experimental support for the use of gene-based and virus-based therapies in reducing myocyte loss during early reperfusion.

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References

- Anversa P, Cheng W, Liu Y, Leri A, Redaelli G, Kajstura J. Apoptosis and myocardial infarction. *Basic Res Cardiol*. 1998; 93(Suppl 3):8–12. [PubMed: 9879436]
- Aouam K, Tissier R, Bruneval P, Mandet C, Berdeaux A, Ghaleh B. Preconditioning of salvaged myocardium in conscious rabbits with postinfarction dysfunction. *Am J Physiol Heart Circ Physiol*. 2005; 288(6):H2763–2769. [PubMed: 15665060]
- Boatright KM, Salvesen GS. Mechanisms of caspase activation. *Curr Opin Cell Biol*. 2003; 15(6):725–731. [PubMed: 14644197]
- Chatterjee S, Stewart AS, Bish LT, Jayasankar V, Kim EM, Pirollo T, Burdick J, Woo YJ, Gardner TJ, Sweeney HL. Viral gene transfer of the antiapoptotic factor Bcl-2 protects against chronic postischemic heart failure. *Circulation*. 2002; 106(12 Suppl 1):I212–217. [PubMed: 12354736]
- Chu G, Luo W, Slack JP, Tilgmann C, Sweet WE, Spindler M, Saupe KW, Boivin GP, Moravec CS, Matlib MA, Grupp IL, Ingwall JS, Kranias EG. Compensatory mechanisms associated with the hyperdynamic function of phospholamban-deficient mouse hearts. *Circ Res*. 1996; 79(6):1064–1076. [PubMed: 8943945]
- Chua CC, Gao J, Ho YS, Xiong Y, Xu X, Chen Z, Hamdy RC, Chua BH. Overexpression of IAP-2 attenuates apoptosis and protects against myocardial ischemia/reperfusion injury in transgenic mice. *Biochim Biophys Acta*. 2007; 1773(4):577–583. [PubMed: 17321613]
- Denault JB, Salvesen GS. Caspases: keys in the ignition of cell death. *Chem Rev*. 2002; 102(12):4489–4500. [PubMed: 12475198]
- Depre C, Kim SJ, John AS, Huang Y, Rimoldi OE, Pepper JR, Dreyfus GD, Gaussin V, Pennell DJ, Vatner DE, Camici PG, Vatner SF. Program of cell survival underlying human and experimental hibernating myocardium. *Circ Res*. 2004; 95(4):433–440. [PubMed: 15242971]
- Deveraux QL, Roy N, Stennicke HR, Van Arsdale T, Zhou Q, Srinivasula SM, Alnemri ES, Salvesen GS, Reed JC. IAPs block apoptotic events induced by caspase-8 and cytochrome c by direct inhibition of distinct caspases. *Embo J*. 1998; 17(8):2215–2223. [PubMed: 9545235]
- Deveraux QL, Takahashi R, Salvesen GS, Reed JC. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature*. 1997; 388(6639):300–304. [PubMed: 9230442]
- Fishbein MC, Meerbaum S, Rit J, Lando U, Kanmatsuse K, Mercier JC, Corday E, Ganz W. Early phase acute myocardial infarct size quantification: validation of the triphenyl tetrazolium chloride tissue enzyme staining technique. *Am Heart J*. 1981; 101(5):593–600. [PubMed: 6164281]
- Giordano FJ, He H, McDonough P, Meyer M, Sayen MR, Dillmann WH. Adenovirus-mediated gene transfer reconstitutes depressed sarcoplasmic reticulum Ca²⁺-ATPase levels and shortens prolonged cardiac myocyte Ca²⁺ transients. *Circulation*. 1997; 96(2):400–403. [PubMed: 9244202]
- Gottlieb RA, Burleson KO, Kloner RA, Babior BM, Engler RL. Reperfusion injury induces apoptosis in rabbit cardiomyocytes. *J Clin Invest*. 1994; 94(4):1621–1628. [PubMed: 7929838]

- Hajjar RJ, Schmidt U, Matsui T, Guerrero JL, Lee KH, Gwathmey JK, Dec GW, Semigran MJ, Rosenzweig A. Modulation of ventricular function through gene transfer in vivo. *Proc Natl Acad Sci U S A*. 1998; 95(9):5251–5256. [PubMed: 9560262]
- Harlin H, Reffey SB, Duckett CS, Lindsten T, Thompson CB. Characterization of XIAP-deficient mice. *Mol Cell Biol*. 2001; 21(10):3604–3608. [PubMed: 11313486]
- Haunstetter A, Izumo S. Toward antiapoptosis as a new treatment modality. *Circ Res*. 2000; 86(4):371–376. [PubMed: 10700440]
- He H, Giordano FJ, Hilal-Dandan R, Choi DJ, Rockman HA, McDonough PM, Bluhm WF, Meyer M, Sayen MR, Swanson E, Dillmann WH. Overexpression of the rat sarcoplasmic reticulum Ca²⁺ ATPase gene in the heart of transgenic mice accelerates calcium transients and cardiac relaxation. *J Clin Invest*. 1997; 100(2):380–389. [PubMed: 9218515]
- Hofer-Warbinek R, Schmid JA, Stehlik C, Binder BR, Lipp J, de Martin R. Activation of NF-kappa B by XIAP, the X chromosome-linked inhibitor of apoptosis, in endothelial cells involves TAK1. *J Biol Chem*. 2000; 275(29):22064–22068. [PubMed: 10807933]
- Holly TA, Drincic A, Byun Y, Nakamura S, Harris K, Klocke FJ, Cryns VL. Caspase inhibition reduces myocyte cell death induced by myocardial ischemia and reperfusion in vivo. *J Mol Cell Cardiol*. 1999; 31(9):1709–1715. [PubMed: 10471354]
- Huang CH, Kim SJ, Ghaleh B, Kudej RK, Shen YT, Bishop SP, Vatner SF. An adenosine agonist and preconditioning shift the distribution of myocardial blood flow in conscious pigs. *Am J Physiol*. 1999; 276(2 Pt 2):H368–375. [PubMed: 9950835]
- Huang J, Ito Y, Morikawa M, Uchida H, Kobune M, Sasaki K, Abe T, Hamada H. Bcl-xL gene transfer protects the heart against ischemia/reperfusion injury. *Biochem Biophys Res Commun*. 2003; 311(1):64–70. [PubMed: 14575695]
- Isner JM. Myocardial gene therapy. *Nature*. 2002; 415(6868):234–239. [PubMed: 11805848]
- Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. *Lab Invest*. 1996; 74(1):86–107. [PubMed: 8569201]
- Kelly KJ, Sandoval RM, Dunn KW, Molitoris BA, Dagher PC. A novel method to determine specificity and sensitivity of the TUNEL reaction in the quantitation of apoptosis. *Am J Physiol Cell Physiol*. 2003; 284(5):C1309–1318. [PubMed: 12676658]
- Kim J, Park J, Choi S, Chi SG, Mowbray AL, Jo H, Park H. X-linked inhibitor of apoptosis protein is an important regulator of vascular endothelial growth factor-dependent bovine aortic endothelial cell survival. *Circ Res*. 2008; 102(8):896–904. [PubMed: 18309102]
- McConkey DJ. Biochemical determinants of apoptosis and necrosis. *Toxicol Lett*. 1998; 99(3):157–168. [PubMed: 9862281]
- Morisco C, Seta K, Hardt SE, Lee Y, Vatner SF, Sadoshima J. Glycogen synthase kinase 3beta regulates GATA4 in cardiac myocytes. *J Biol Chem*. 2001; 276(30):28586–28597. [PubMed: 11382772]
- Olivetti G, Capasso JM, Meggs LG, Sonnenblick EH, Anversa P. Cellular basis of chronic ventricular remodeling after myocardial infarction in rats. *Circ Res*. 1991; 68(3):856–869. [PubMed: 1742871]
- Riedl SJ, Renatus M, Schwarzenbacher R, Zhou Q, Sun C, Fesik SW, Liddington RC, Salvesen GS. Structural basis for the inhibition of caspase-3 by XIAP. *Cell*. 2001; 104(5):791–800. [PubMed: 11257232]
- Roy N, Deveraux QL, Takahashi R, Salvesen GS, Reed JC. The c-IAP-1 and c-IAP-2 proteins are direct inhibitors of specific caspases. *Embo J*. 1997; 16(23):6914–6925. [PubMed: 9384571]
- Salvesen GS. Caspases: opening the boxes and interpreting the arrows. *Cell Death Differ*. 2002; 9(1):3–5. [PubMed: 11803369]
- Saraste A, Voipio-Pulkki LM, Parvinen M, Pulkki K. Apoptosis in the heart. *N Engl J Med*. 1997; 336(14):1025–1026. author reply 1026. [PubMed: 9091792]
- Stehlik C, de Martin R, Kumabashiri I, Schmid JA, Binder BR, Lipp J. Nuclear factor (NF)-kappaB-regulated X-chromosome-linked iap gene expression protects endothelial cells from tumor necrosis factor alpha-induced apoptosis. *J Exp Med*. 1998; 188(1):211–216. [PubMed: 9653098]

- Stennicke HR, Ryan CA, Salvesen GS. Reprieval from execution: the molecular basis of caspase inhibition. *Trends Biochem Sci.* 2002; 27(2):94–101. [PubMed: 11852247]
- Yamamoto S, Seta K, Morisco C, Vatner SF, Sadoshima J. Chelerythrine rapidly induces apoptosis through generation of reactive oxygen species in cardiac myocytes. *J Mol Cell Cardiol.* 2001; 33(10):1829–1848. [PubMed: 11603925]
- Yang G, Meguro T, Hong C, Asai K, Takagi G, Karoor VL, Sadoshima J, Vatner DE, Bishop SP, Vatner SF. Cyclosporine reduces left ventricular mass with chronic aortic banding in mice, which could be due to apoptosis and fibrosis. *J Mol Cell Cardiol.* 2001; 33(8):1505–1514. [PubMed: 11448138]
- Zhao ZQ, Vinten-Johansen J. Myocardial apoptosis and ischemic preconditioning. *Cardiovasc Res.* 2002; 55(3):438–455. [PubMed: 12160941]

Figure 1. XIAP protein expression in Ad.XIAP transfected rabbit myocardium. Note that the enhanced XIAP protein expression in rabbits was sustained 3 weeks after Ad.XIAP gene delivery.

Figure 2.

A. A representative heart slice from a control rabbit heart demonstrating the area at risk and the myocardial infarction. The area not at risk is indicated by blue dye and the area at risk is unstained. Myocardial infarction is indicated by the pale tissue contained within the area at risk, which, in this slice, is contained primarily within the subendocardium. B. The myocardial infarct (MI) size expressed as a percentage of area at risk (AAR) and the size of AAR, expressed as a percentage of left ventricle (LV). Ad.XIAP transfected hearts (n=5) demonstrated a significant reduction of myocardial infarct size compared to control hearts (n=7). *, $p < 0.05$.

Figure 3. Light microscopy of paraffin section with Mallory's trichrome stain, demonstrating relative number of myocytes remained survived (Red) in the infarcted (Blue) region in XIAP transfected heart (B) compared to control heart (A).

Figure 4.

A, Representative TUNEL stained cardiomyocytes in the ischemic region from control (left) and Ad.XIAP (right) rabbit hearts (original magnification x40). Upper panel: myocytes were stained TUNEL (green), DAPI (blue), and WGA (red); Lower panel: myocytes were stained TUNEL (green) and DAPI (blue). Arrows indicate TUNEL positive myocytes. Use of counterstaining with WGA localized apoptosis within the cardiomyocytes. B, Summarized results of % of TUNEL-positive cardiomyocyte nuclei in peri-infarct zone (IZ, left) and non-ischemic zone (NIZ) from Control (n=5) and Ad.XIAP transfected (n=4) rabbits, demonstrating markedly decrease in apoptosis in XIAP transfected hearts compared to control hearts. TUNEL, terminal deoxynucleotide transferase-mediated dUTP nick end labeling; DAPI, 4,6-diamidino-2-phenylindole; WGA, rhodamine wheat germ agglutinin; IZ: ischemic zone; NIZ: non-ischemic zone. *, $p < 0.05$.

Figure 5.

A, Western blots for XIAP and cleaved caspase-3 protein in Control (n=4) and Ad.XIAP transfected (n=4) rabbit myocardium. B, Summarized results of the expression of XIAP (left) and cleaved caspase-3 (right) protein, demonstrating reciprocal relationship between XIAP and cleaved caspase-3 protein expression. *, $p < 0.05$.