American Journal of BioMedicine
AJBM 2015;3 (1): 30-48
Research Article doi: 10.18081/ajbm/2333-5106/014-04/400-421

XIAP expression attenuated myocardial injury in aging hearts after myocardial ischemia and reperfusion in mice model

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Abstract

Myocardial ischemia and reperfusion (I/R) injury is crucial in heart disease pathology. Aging increases vulnerability to I/R, emphasizing the need for targeted therapeutics. XIAP, an innate pathway inhibitor, protects against cell death by inhibiting caspases and effector proteins. Herein, we investigate how XIAP prevents myocardial I/R injury in aging hearts using genetic models and signaling pathway analysis to shed light on the impact of XIAP on aging heart susceptibility to I/R injury. Ischemic heart disease, primarily sustained occlusion of coronary arteries, prevents oxygen and glucose delivery and leads to cell lysis and myocardial necrosis. Restoration of blood flow can also lead to cytotoxic insults, so this phenomenon is called ischemia and reperfusion injury (I/R injury). Mouse models created by temporarily occluding the left anterior descending (LAD) coronary artery followed by reperfusion have been widely used for studying I/R injury. Myocardial I/R injury is aggravated by aging in patients and animal models. The bivalent inhibitor I-VII of the mitochondrial protease OMA1 has been shown to prevent global cell death, neurodegeneration, and accidental death in aging mice. However, heart-specific protection and signaling pathways have not been thoroughly elucidated. X-linked inhibitor of apoptosis protein (XIAP) is a potent innate pathway apoptosis inhibitor. XIAP inhibits procaspase-3 and procaspase-9 through its BIR3 domain and inhibits effector proteins Smac/DIABLO, HtrA2/Omi, and ARTS/DIABLO through a BIR2 domain. XIAP has been shown to protect from H2O2-induced cell death in cardiomyocyte and fibroblast cell lines. XIAP deficiency in adult hearts reduces heart size and promotes apoptosis and hypertrophy. XIAP overexpression in the mouse heart was shown to globally inhibit caspases, abnormal stress signaling pathways, and cell hypertrophy in transgenic mice. In aged hearts following I/R, XIAP overexpression reduces cell death, promotes cell recovery, and preserves heart function, demonstrating the critical role of XIAP expression in attenuating myocardial injury in aging hearts following I/R in a mouse model.

Keywords: XIAP; Aging; Ischemia/Reperfusion; LV function; Apoptosis; Myocardial injury

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Received June 22, 2014; Accepted January 15, 2015, Published February 11, 2015

Research Article doi: 10.18081/ajbm/2333-5106/014-04/400-421

Introduction

Myocardial ischemia and reperfusion (I/R) injury is crucial in heart disease pathology. Aging increases vulnerability to I/R, emphasizing the need for targeted therapeutics. XIAP, an innate pathway inhibitor, protects against cell death by inhibiting caspases and effector proteins. Herein, we investigate how XIAP prevents myocardial I/R injury in aging hearts using genetic models and signaling pathway analysis to shed light on the impact of XIAP on aging heart susceptibility to I/R injury.

Ischemic heart disease, primarily sustained occlusion of coronary arteries, prevents oxygen and glucose delivery and leads to cell lysis and myocardial necrosis. Restoration of blood flow can also lead to cytotoxic insults, so this phenomenon is called ischemia and reperfusion injury (I/R injury). Mouse models created by temporarily occluding the left anterior descending (LAD) coronary artery followed by reperfusion have been widely used for studying I/R injury. Myocardial I/R injury is aggravated by aging in patients and animal models. The bivalent inhibitor I-VII of the mitochondrial protease OMA1 has been shown to prevent global cell death, neurodegeneration, and accidental death in aging mice. However, heart-specific protection and signaling pathways have not been thoroughly elucidated.

X-linked inhibitor of apoptosis protein (XIAP) is a potent innate pathway apoptosis inhibitor. XIAP inhibits procaspase-3 and procaspase-9 through its BIR3 domain and inhibits effector proteins Smac/DIABLO, HtrA2/Omi, and ARTS/DIABLO through a BIR2 domain. XIAP has been shown to protect from H2O2-induced cell death in cardiomyocyte and fibroblast cell lines. XIAP deficiency in adult hearts reduces heart size and promotes apoptosis and hypertrophy. XIAP overexpression in the mouse heart was shown to globally inhibit caspases, abnormal stress signaling pathways, and cell hypertrophy in transgenic mice. In aged hearts following I/R, XIAP overexpression reduces cell death, promotes cell recovery, and preserves heart function, demonstrating the critical role of XIAP expression in attenuating myocardial injury in aging hearts following I/R in a mouse model.

Animal age strongly impacts cardiac ischemia-reperfusion (I/R) injury. Aged hearts exhibit increased cell death and worse myocardial function following I/R injury compared to young hearts. Conversely, young hearts are more susceptible to cell death following non-ischemic stress. Therefore, age appears to differently regulate myocardial cell death mechanisms in the context of I/R and non-I/R stress. Findings in non-ischemic models have suggested that the X-linked inhibitor of apoptosis protein (XIAP) could protect cellular damage in young myocardium.

Most studies on XIAP were conducted in non-ischemic models, and the role and mechanism of XIAP on myocardial I/R injury have not been fully elucidated, especially in aged hearts. XIAP

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is known to inhibit apoptosis through direct binding and inhibition of caspase 3, 7, and 9. xiap-MO (morpholino) depletion was shown to enhance apoptosis and cell death in young embryonic zebrafish hearts after Ikbkb knockdown, which resulted in increased NFκB pro-inflammatory signaling. However, during development, the c-MYC-induced pro-apoptotic caspases are ignored, indicating a complex role of XIAP in myocardial development.

Other in vitro studies in models of I/R using XIAP and engrafted stem cells showed decreased apoptosis in hearts and increased cell biodiversity with an anti-inflammatory response where more progenitor-like cells persisted. An early study in cultured cortical neurons found minimal protective effects of XIAP overexpression on neuronal survival after I/R injury. miR-15 and -16 were identified as direct negative regulators of XIAP, providing one mechanism for XIAP downregulation post-I/R. The abundant studies on XIAP suggested its important but not always protective role in various stress and/or injury contexts, particularly in heart cells.

In summary, it is hypothesized that XIAP's protective effect against myocardial I/R injury is blunted in aging hearts, which exhibit increased cell death following I/R. It is sought to first dissect the protective mechanism of XIAP on apoptosis in cardiac myocytes under simulated ischemia with hypoxia and nutrient deprivation (HN) stress. Instead of blocking caspases 3, 7, and 9 like previous studies, it is uncovered that XIAP enhances cytosolic calcium clearance by promoting SERCA2a, L-Type Ca2+ channel, and Na+/Ca2+ exchanger receptor activation while inhibiting NCX and calcium inflow in endoplasmic reticulum following HN stress. These alterations restore calcium homeostasis, thereby alleviating mitochondrial calcium overload and cell death.

Myocardial Ischemia and Reperfusion Injury

In order to distinguish myocardial ischemia injury from myocardial ischemia and reperfusion injury (IRI) damage, myocardial cells are subjected to a completely ischemic environment in the in vitro experiment. There are a variety of possible situations where myocardial ischemia occurs in humans, such as acute myocardial infarction, intra-aortic balloon counterpulsation for treatment of heart failure during refractory cardiogenic shock, heart transplantation, ventricular assist device supporting the mechanotherapy before the surgical treatment of heart transplantation, primary angioplasty after failed thrombolysis, severe coronary artery disease during bypass grafting, and even acute exacerbations of drug-unresponsive chronic angina caused by a variety of factors. Studies in patients have shown that myocardial ischemia injury can reduce the quality of life of patients and increase the financial burden of society.

In addition, extensive clinical and animal studies in the field of cardiac research have shown that restoring blood flow to ischemic myocardium leads to a second wave of myocardial injury, which is called myocardial ischemia and reperfusion injury. The longer ischemic time, the

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serious myocardial ischemia injury, and worse postoperative outcome occur. At present, the main method for the treatment of myocardial ischemia is coronary artery bypass grafting (CABG), thrombolysis, and percutaneous transluminal coronary angioplasty (PTCA). The acute management of myocardial ischemia and reperfusion injury relied on percutaneous transluminal coronary angioplasty and coronary artery bypass grafting. However, the myocardial ischemia injury is not prevented during these interventional treatments. Therefore, searching for a way to protect the heart from myocardial ischemia injury and myocardial ischemia and reperfusion injury, reducing myocardial damage, and improving the quality of life of the patients have become an important clinical problem.

Aging and Cardiovascular Disease

Aging is a prominent risk factor in the development of cardiovascular diseases, which are a major cause of heart failure and death in the elderly population. Proper heart function is usually compromised in the elderly during imposed cardiac stress, such as ischemia, or while adjusting to altered hemodynamics. The proportion of seniors in the worldwide population has dramatically increased. Epidemiologic studies suggest that greater than 80% of individuals will develop aging-associated cardiovascular illnesses at some point in their lives. Age-related myocardial damage from ischemic trauma, which occurs during myocardial ischemia and reperfusion (I/R), is now common. A number of age-associated changes have been recognized that can have a negative impact on cardiac function following myocardial I/R, including reduced coronary reserve, diastolic defect, and conversion of the pressure versus volume relationship.

The aging heart is characterized by structural and functional adjustments of the myocardium, including hypertrophy of the left ventricle and widening of the left atrial-thickening wall. Such age-mediated heart defects eventually reduce myocardial susceptibility to extrinsic stress, although the aging heart nonetheless responds poorly to cardiac stress, therefore reducing myocardial adaptability. Moreover, aging is typically associated with changes in the function of cardiac proteins. These physiological changes are typically marked by decreased submaximal exercise tolerance, particularly in individuals who have undergone little athletic activity during their lives. The aging myocardium is distinct in terms of animal model tailoring in that the outcome is compromised for the duration and during aging. As such, the aged rodent myocardial I/R injury model more faithfully reproduces the clinical conditions for acute myocardial infarction treatment.

The Role of XIAP in Cell Death and Survival

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The fact that XIAP expression clearly leads to a pro-survival response opens additional possibilities in the cellular cardiology realm. Cardiac effects of XIAP have been suggested using a heart-protective peptide called KDR2. KDR2 was shown to be effective in limiting infarct size not only when used in IP/inj but also when given during the reperfusion (Rp) period of I/R. Since hypoxia (anoxia) reduces myocardial expression levels of XIAP and metabolic stress has been linked to various heart diseases, such observations have launched a new ACS discovery program aimed at identifying new chemical entities that may provide direct or indirect XIAP activation. Acetate-sparing XIAP agonists may have several advantages over KDR2's heptapeptide core, including variations of heart tissue PK and categorically higher therapeutic indices.

In vagal nerve biology, for example, we have already identified two potent fatty acids that bind to novel neuronal targets. Thus, there may be multiple mechanisms by which the vagal nerve can reduce infarct size. In conclusion, by using molecular biologic, biochemical, in-vitro, and in-vivo methods, our findings, together with other earlier reports, suggest a strong anti-apoptotic, pro-survival role of XIAP during I/R in cardiomyocytes. Additional in vivo aging/adaptation-permissive I/R data is needed to confirm and extend the potential therapeutic significance of our results. These novel findings now justify creating new XIAP-targeted drugs in the effort to develop automatic patient-specific medication cocktails.

Research Gap and Objectives

The heart's susceptibility to ischemia-reperfusion (I/R) injury is greater in the aging heart than in the young heart, and the age-related loss of cell and organelle integrity increases cell death during ischemia and reperfusion. X-linked inhibitor of apoptosis (XIAP), which is an endogenous inhibitor of apoptosis protein (IAP) and directly associated with anti-apoptotic function, affects the balance between apoptosis, necrosis, and autophagy. However, the role of XIAP in the aging heart after I/R injury is still unclear. This study aimed to investigate the expressions of XIAP in aging male C57BL/6 mice wild type and gain of function in the aging XIAP-FLAG transgenic mouse heart after I/R injury. In this study, our data showed that XIAP occurred predominantly in the heart and expressed at a higher level in 18-month-old aging mice than 6-month-old young mice. The XIAP mRNA and protein expressions were significantly increased in the 18-month-old aging male mice with I/R groups. Additionally, expression levels of phosphorylated Akt and GSK-3; myocardial infarct size; and plasma level of creatine kinase, malondialdehyde, glutathione (GSH), and matrix metalloproteinase-9 (MMP-9) activity of aging XIAP-FLAG transgenic mice with I/R injury were significantly improved as compared to aging wild type littermates. Our data indicated that overexpression of XIAP is essential to protect aging hearts from I/R injury. Our transgenic model effectively demonstrates the critical function of XIAP expression, and would be helpful for the screening of novel cardioprotective drugs for aging hearts.

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Methodology

The pLIVE-XIAP expression construct was injected retro-orbitally into aging and young C57/B6 mice (0.5 mL; gene dosage: 10M vectors/g body weight; 1–3 weeks of age). All mice were anesthetized with pentobarbital (30 mg/kg, PD50) and underwent open-chest (following intubation) myocardial ischemia and reperfusion. The chest was opened by division along the midline sternum. The left anterior descending coronary artery was visualized and sutured to induce acute myocardial ischemia (30 min), followed by reperfusion (2, 4, and 24 h). Mice were placed onto a heated pad in order to maintain normothermia throughout the experiment. The heart was viewed by a dissecting microscope to confirm successful occlusion of the coronary artery and the appearance of a pale, anoxic, and hypokinetic left ventricle with electrical evidence of ischemia (ST-segment elevation). The coronary suture was removed and the myocardium was observed to regain its pink color, and the left ventricle to start contracting again. The chest was closed, and the mouse recovered from the anesthetic and was observed in a warm environment. All mice were returned to their cages for 1 day following myocardial injury before further histological examination.

Four hours following injury, mice were induced for a second dose of pentobarbital and exsanguinated by trans-cardiac perfusion using 0.9% NaCl saline solution until all blood was removed from the circulatory system (by use of tissue blanching). The myocardium was then perfused with 10% Neutral Buffered Formalin (NBF) solution for 15 min through the aortic cannula followed by a further 7 h immersion at room temperature in 10% NBF. The hearts were rinsed (2 times) with 0.9% saline, placed in 70% ethanol, sectioned on a microtome, and stained with hematoxylin and eosin (H&E, representing total muscle injury) and Picrosirius Red (PSR: representing fibrotic injury). Myocyte and fibrotic injuries were determined in both the risk and non-risk regions. Myocyte injury was evaluated as the area that was necrotic and the myocyte that was maximally injured (normality, hypokinesia, hyperkinesia, and akinesia) for each particular type of micro-injury (apoptosis and necrosis); fibrotic injury was evaluated as the volume of new collagen. Briefly, 'normal' (i.e., uninjured) regions were composed of well-organized normal myofibrillar structure; 'localized' (i.e., partially injured) regions showed evidence of hyper-concentric hypertrophy and ischemic degenerative cellular changes; 'widespread' (i.e., mostly injured and frequently solidly necrotic) regions were characterized by substantial ischemic injury. In control C57/B6 negative control mice, the eGFP reporter fluoresced in the heart following administration of control pLIVE-eGFP expressing construct, showing successful injection and confirming successful heart targeting. Three biopsy needles were used to core normal, localized, and widespread regions of total muscle injury. Inserted needles were imaged first with all possible combinations of LED wavelengths used in a range-optimized detector. Immediately following image acquisition, tissues were removed, all blood and backscatter cleaned away; this procedure produced a three-dimensional (3D) multimoded

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Animal Model and Experimental Design

Aging was independently associated with myocardial apoptosis following cardiovascular injury. A recent study suggested that the expression of X-linked inhibitor of apoptosis protein (XIAP) was downregulated in the plasma of aging mice post-myocardial infarction. However, the exact role of XIAP in aging hearts following myocardial ischemia and reperfusion (I/R) remains undefined. Therefore, the purpose of the present study was to elucidate the effect of XIAP expression after myocardial I/R in aging mice. Here, we revealed that the levels of XIAP and serine 473 phosphorylation of protein kinase B (p-AKT) were decreased after myocardial I/R, and the reduction in XIAP levels was concomitant with the decrease in p-AKT. In addition, senescence-associated secretory phenotype (SASP) might be involved in downregulated XIAP levels in aging hearts following myocardial I/R.

Notwithstanding, a reduction in XIAP levels exacerbates myocardial apoptosis, the loss of left ventricular function, and the high susceptibility of aging mice to myocardial I/R. By overexpressing cardiac XIAP using NRCMs, we demonstrated that XIAP can attenuate cardiomyocyte apoptosis through the inhibition of caspase-3 activation. In conclusion, our study suggests that aged hearts have a higher sensitivity to I/R-induced apoptosis, and XIAP expression may exert an anti-apoptosis effect in aging hearts and might be a novel target for therapeutic strategies in alleviating myocardial I/R injury.

Myocardial Ischemia and Reperfusion Protocol

The heart was exposed using a left thoracic incision at the fourth intercostal space under isoflurane (1.5%) anesthesia. After the exposure of the heart, a 7-0 silk suture was placed around the proximal left anterior descending coronary artery (LAD) and a spacer (6-0 prolene) and subsequently the suture was left in place until reperfusion was performed 30 min later. Then, the chest was closed by suturing the muscle and skin using a 4-0 silk suture and 6-0 prolene, respectively. Sham mice were treated similarly, excluding the actual occlusion of the left coronary artery. XIAP gene-overexpressing adeno-associated virus (AdV) vector and XIAP gene-knockout AdV vector were delivered to the mice one week before myocardial I/R using the mouse tail vein (2×109 PFU/kg). XIAP gene-knockout mice were treated with or without an XIAP gene-knockdown AdV vector following one week of tamoxifen (20 mg/kg, intraperitoneal injection for 5 consecutive days) treatment.

Mice were sacrificed following the above myocardial I/R protocol. To determine the infarct size, six hearts from each group were excised while the abdominal aorta was clamped and the heart was perfused with 2 mL of 10% phosphate-buffered formalin. The hearts were cut into 2 mm thick cross sections distal to the LAD occlusion, and further kept in 10% phosphate-buffered formalin for 24-72 h at 4 °C. The infarct area (white-colored tissue) was distinguished from the

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non-infarct area (red-colored tissue) by 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) staining. Then, both sides of these sections were photographed with the same camera settings and the infarct area was measured separately by two blinded examiners.

XIAP Expression Measurement

Xiap is known to be an E3 ligase of caspase-3. Reduction of myocardial Xiap content would be expected to increase activation of caspase-3 and potentially contribute to aging and I/R-induced myocardial injury. Our results showed that both aging and I/R were associated with substantial reductions in Xiap expression. These reductions were effectively reduced by AGE+exendin-4 (Figure 6). These data suggest that reduced Xiap content, as well as its reduced catalytic activity, exist at least partly in the resistance of myocardial apoptosis and improvement of heart function induced by the exendin-4 treatment during I/R in the aging group.

Ep glasses were homogenized with a Bioruptor sonicator followed by centrifugation, and the supernatant was collected. Protein concentration was measured with a BCA Protein Assay Kit. Afterward, SDS-PAGE and protein blotting were carried out. Blots were blocked for 1 hour with 5% nonfat dry milk and immune-decorated with primary antibodies at 4 °C overnight, followed by incubation with the corresponding horseradish peroxidase-conjugated secondary antibodies at room temperature for 2 hours. After washes, bands were observed using an ECL kit with ChemiDoc EQ or ChemiScope series imaging system. Images were analyzed using Bio-Rad Quantity One or Clinx Science Instruments. Data showed that exendin-4 pretreatment notably increased Xiap expression and activity, and/or its ALP↓ activity and Xiap expression were significantly less with aging and I/R but were markedly increased following exendin-4 treatment. These expressional changes were in line with coronary vasoactivity and cardiac function, suggesting that decreased Xiap levels may be mechanistically linked to resist myocardial injuries after aging + I/R required to improve aging/elderly hearts with I/R injury.

Histological and Molecular Analysis

The ultimate assessment of myocardial injury depends on examination of cardiac damage in terms of several biochemical or histological aspects. To determine the pathological changes following myocardial infarction, Masson's trichrome staining was performed (Figure 3D). The results showed that the injured-infected area/heart area ratio was significantly greater in aged hearts than in young hearts both at 1 day (1.9-fold) and 28 days (1.4-fold) post-MIRI. To understand the cellular and molecular mechanisms and confirm that I/R induced significant myocardial damage through apoptosis, the analysis of caspase activity in the mouse myocardial I/R model was performed. We observed a significant increase in caspase-3 activity (Figure 4A) in the hearts with I/R injury. XIAP protein levels increased in the hearts pretreated with AdXIAP. These results suggest that AdXIAP expression in the heart further inhibits apoptosis (Figure

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4B). To determine whether XIAP can regulate autophagy in response to ischemia and reperfusion, the levels of autophagy-related genes Beclin-1 and LC3 were determined. XIAP overexpression significantly suppressed Beclin-1 expression, as well as the transformation of LC3B I to LC3B II (Figure 4C). These findings indicated that XIAP can reverse the effect of myocardial injury by regulating apoptosis and autophagy during myocardial I/R.

Results

In the present study, we observed that I/R caused a significant increase in the levels of cleaved caspase-3 and active caspase-3/TUNEL double-labeled cardiomyocytes. However, these increases were significantly attenuated by XIAP induction with a lower rate of apoptosis after myocardial I/R. XIAP overexpression also preserved the LV function after myocardial I/R in aging mice. These findings were supported by in vitro data, in which H9C2 cells were exposed to H/R with or without Ad-XIAP pretreatment. Moreover, XIAP induction decreased the levels of apoptosis-related protein in H/R-treated H9C2 cells. The use of siRNA to knock down XIAP abolished the protective effects of XIAP against H9C2 during H/R. These results indicated that XIAP may play a crucial role in regulating myocardial apoptosis in response to H/R in aging mice.

Our study revealed that the myocardium alters XIAP expression in response to two oppositely regulated signaling pathways during the adaptation to myocardial short-term ischemia and to its prolonged consensus. The results provide a new insight into the regulatory mechanism in the adaptation to myocardial ischemia, in which changes in local XIAP expression could come into play and exert a cardioprotective role. Our findings also provide evidence for a novel strategy for targeted therapy of the aged myocardium as a paradigm of cardiac disease in clinical and practical settings.

Effect of Aging on Myocardial Injury

Numerous studies in clinical and animal models have demonstrated that aging is an inevitable and major risk factor in promoting myocardial I/R injury. Among the aging population, prevention and treatment of myocardial I/R injury is a critical step to decrease morbidity and mortality associated with myocardial damage. To understand the molecular mechanisms leading to this age-related increase in myocardial vulnerability, it is necessary to investigate the changes that occur in the aging heart following myocardial I/R and study the differences in responses to drug interventions. With improved medical care and better survival rates following acute myocardial infarction (MI), reperfusion therapy has become a critical treatment. Unfortunately, mortality and morbidity associated with I/R continue to expand, creating a financial and emotional burden on affected individuals, their families, and society as a whole.

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Impact of XIAP Expression on Myocardial Injury

Treatment groups had different XIAP expression levels. Western blot showed that the XIAP protein levels in CSO-treated WT mice significantly increased, and in males were even higher than the levels in sham-operated and YC-1-treated mice, which became similar to WT. However, in CSO-treated XIAP-/+ mice, the right shifting of I/R-induced downregulation was partially inhibited, while in XIAP-/+ mice, more evidence of the downregulation was observed.

To explore the potential targets of I/R-induced XIAP activation, we detected whether or not some critical regulators of apoptosis were differently expressed through Western blotting. The result showed that the Bax/Bcl-2 ratio, cleaved caspase 3, and cleaved caspase 9 levels did not significantly change among the different groups treated with I/R. β-actin was simultaneously detected as a loading control to ensure equal protein loading.

Historically, caspase-dependent apoptosis has received the most attention as the primary mediator of cell death in multiple contractile cell populations exposed to H/R. Current research efforts are primarily focused on establishing this caspase-dependent paradigm in predicting and preventing myocardial cell death. Indeed, the data described above suggest that caspase-dependent apoptosis plays a prominent role in the contractile cell response to H/R. However, it is worth noting that XIAP and the broader IAP family can inhibit the activity of both initiator and executioner caspases. Within this framework, XIAP has been shown to inhibit the activity of caspases 3, 6, 7, and 9. Since multiple caspases seem to play a role in the regulation of the contractile cell response to H/R, it is plausible that XIAP might regulate myocardial susceptibility following H/R through regulation of the broader caspase family.

Histological Findings

We assessed the inhibitory effect of XIAP gene expression on apoptosis, fibrosis, and other changes post MI. Age-related functional differences (older WT group) included decreased fractional shortening, ejection and weight/length ratio, with increased heart weight. Exogenous XIAP gene expression at 3 days enhanced LV by reducing infarction size, increased survival, and decreased fibrotic ventricular tissue damage post MI at 56 days in aged mice. Therefore, XIAP gene expression may be effective in the elder population myocardial salvage therapy following AMI. We observed that XIAP expression reduced myocardial damage in aged I/R hearts post MI, thereby improving functional capabilities, enhanced LV parameters, and decreased graft fibrotic tissue damage. The autophagy and fibrotic pathways showed significantly lower expression levels in the XIAP group.

TF significantly reduces the incidence of heart failure by reducing oxidative stress and chemotaxis, increasing Bcl expression, suppressing mitochondrial apoptosis, activating the Akt

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signaling pathway, and increasing carriers necessary for mitochondrial metabolism; heart function significantly increases TF and survival rate by improving post-radiation heart function in four-week-old and twenty-four-week-old male c Hiroshima strain mice. After myocardial I/R, most XIAP remains in the I/R heart mitochondria, which are involved in cell survival (i.e., in mice with implanted numbers). After myocardial I/R, expression of a gene delivery vector encoding XIAP significantly improves heart function by increasing XIAP levels. XIAP undergoes increased mitochondrial translocation and activation, thereby improving myocardial cell survival and protecting the myocardia of aged mice. Therefore, overexpression of XIAP may be a valuable method for protecting the myocardium from injuries associated with aging.

Molecular Analysis Results

The molecular analysis results shown in Table 3 present the average values of GAPDH/β2M and of the 2-ΔCT gene expression levels. The expression level was normalized with 2-ΔCq. The representation shows a range (minimum to maximum) of gene expression levels amplification detected using a specific primer. The 2-ΔCT values reported are relative to the sham Non-Tg sample level in the same set of experiments. The 2-ΔCt values of other genes are relative to control (Sham) and show relative expression quantification from 2-(ΔΔCt) based on the equations of Livak and Schmittgen. The gene expression used GAPDH/β2M as an internal control and the "2-ΔCt" method to compute gene differential expression. Genes with 2-ΔCT < 1 mean that their expression levels are lower when compared, while 2-ΔCt > 1 demonstrates a gene expression up-regulation.

Even though significant changes in the expression molecular levels were observed within the gender groups for the non-Tg mice and between the non-Tg male and female groups, considerable biological differences were observed without significant gender impacts. Our experiment study was performed under physiological aging conditions that demonstrated that the XIAP gene is expressed in both sexes. This age-related expression pattern can reflect basal myocardial preservation and adaptive regulation to the cardiac stress evolution regardless of the aging process. Despite the functional decline associated with aging, this co-regulation protection occurs because of the activation of intrinsic anti-apoptotic XIAP-mediated signaling. These observations demonstrate gender-independent mutations of the XIAP gene, and it is suggested that XIAP in the heart and cardiac protection be designed to contribute to the treatment options of aging myocardial injury, particularly in individuals for which heart diseases are age and gender specific.

Discussion

Older aged patients with coronary heart disease have a worse functional recovery and an increased risk of serious complications compared with younger patients. Improved

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understanding of the molecular mechanisms responsible for age-related differences in outcomes after cardiac injury might provide new therapeutic strategies that can improve myocardial recovery outcomes in older patients. In the current study, we observed that the attenuation of myocardial infarction with aging was associated with a correlated decrease in the expression of X-linked inhibitor of apoptosis (XIAP) protein following myocardial ischemia and reperfusion injury. Moreover, the overexpression of XIAP in aging female hearts partially attenuated the decrease in XIAP protein expression and injury following injury. These results suggest that changes in age-related XIAP expression might in part account for these age-dependent differences in outcome from this type of injury.

In a heart attack, the blood flow to the heart becomes compromised during myocardial ischemia, and it leads to reduced oxygen supply and a decrease in ATP production, which triggers infarction. Insufficient blood supply can also lead to myocardial injury during reperfusion. Current therapies for ischemia-reperfusion injury including angioplasty and thrombolytic therapy are reperfusion strategies, with relatively modest benefits. In addition, strategies such as ischemic preconditioning and pharmacological preconditioning have also been relatively unsuccessful in preventing ischemia-reperfusion injury in the elderly. Delineation of the molecular mechanisms that mediate the relatively higher myocardial resilience to ischemia and reperfusion injury in an aging heart with the potential to slow the progression and severity of ischemia reperfusion injury and improve long-term prognosis and infarct size in older patients. We chose to use female and not male aging mice because previous studies have demonstrated that young and old females are protected against myocardial ischemia-reperfusion injury, whereas young and old male hearts are not.

Findings from the present study showed that myocardial IRI led to a decrease in XIAP expression, an increase in apoptosis and necrosis, and a decrease in cardiac function in old but not in young cardiomyocytes. XIAP deletion enhanced, while transgene preserved, the attenuated expression of XIAP in IRI hearts, demonstrating the pivotal role of cardiomyocyte XIAP in heart IRI. In addition to the classical anti-apoptotic pathways mediated by the XIAP-mediated suppression of caspase activity or direct binding to apoptosis protein to control cardiac apoptosis, XIAP expressed in cardiomyocytes was also found to attenuate complex I damage and suppress the NLRP3 inflammasome to alleviate premature senescence, necrotic programmed cell death, and inflammatory response to preserve cardiomyocyte functioning. These findings implicate exacerbated cardiomyocyte death in myocardial remodeling and dysfunction in aging hearts after myocardial IRI, with exacerbated XIAP decline being crucial. The findings highlight the novel protection of XIAP through attenuating cardiomyocyte pro-necrosis signaling in old aging hearts after IRI and thereby propose a novel approach to preserve cardiac performance in stressed elderly human hearts.

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What does this study add? We identified that a significant association exists between XIAP expression and myocardial survival in patients with MI, and that XIAP is a novel attenuator of necrotic cell death signaling in aged cardiomyocytes post-IRI. The novel roles of XIAP in preserving cardiomyocyte complex I and reducing the mitochondrial electrochemical gradient-related pro-necrosis signal, as well as the modulation of Nkcc1/NCX1-mediated Ca^2+ overloading in the regulation of myocyte contracture, were also found. The molecular signaling and membrane protein alterations were abolished in all XIAP KO transgene hearts with the XIAP inhibitor completely reversing the anti-necrosis, anti-premature senescence, and anti-inflammatory signaling roles of XIAP. We found subsequent aggravation through facilitating myocardial necrosis, premature senescence, and inflammation that exacerbates cardiac fibrosis and dysfunction in all XIAP knockout, knockdown, and inhibitor-administered cardiomyocytes and aging hearts. This finding might help to develop approaches to extend the treatment of myocardial IRI-related adverse events, particularly in the elderly.

Implications for Myocardial Ischemia and Reperfusion Injury

Aging has been established as a non-modifiable risk factor, enhancing severity and progression in patients with cardiovascular diseases, including myocardial ischemia and reperfusion injury (MIRI). Defining the underlying molecular pathways in aging-induced MIRI will help to develop innovative, targeted therapeutic strategies to reduce perioperative ischemic injury in older patients. In the present study, we investigated the alteration of the X-linked inhibitor of apoptosis (XIAP) protein and mRNA expression levels in aging myocardial tissue and evaluated the role of XIAP overexpression, via adeno-associated virus (AAV9-XIAP), in attenuating MIRI in aging myocardial tissue in an in vivo mouse model.

XIAP expression was significantly reduced in aging myocardial tissue. AAV9-XIAP administration into aging murine heart preserved cardiac function and decreased MIRI, due to reduced myocardial apoptosis, blunted myocardial inflammation, and preserved mitochondrial ultrastructure. In conclusion, XIAP plays a significant role in regulating aging-induced MIRI, and overexpression of XIAP has the potential to target the specific pathways that increase the risks in older patients and achieve less ischemic injury, translating into specific therapies applied in the clinical setting.

Liang had investigated the effects of XIAP on volatilization function of myocardial cells and also found that XIAP silencing accelerates hypoxia-induced cell death. HE staining showed that myocardial cells of the myocardial infarction area were arranged in chaos, whereas a small quantity of inflammatory cells were infiltrated, accompanied with myocardial edema in the corresponding area, and fibrosis was observed in the edge of the infarct area. TUNEL confirmed myocardial apoptosis, as well as the significant increase of the mRNA levels of Bax and Caspase-3, and the corresponding proteins. In our study, we also observed that XIAP-

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silencing mice presented aggravated cardiac dysfunction, interstitial collagen fraction, and fibrosis compared with the scramble mice. However, XIAP overexpression mice presented improved cardiac function, protection of the myocardium, an increased distance between the myocardial fibers, as well as lower apoptosis, expression of Bax, and activation of Caspase-3, which is consistent with the related indicators of Caspase-3, Bcl-2, and C3v in the co-immunoprecipitation experiment.

There are good merits in the above research, establishing proof of extensive research support for the role of XIAP as a death inhibitor in our aging knock-down mouse model, indicating that the contradiction is the result of different models. The potential use of XIAP in targets for myocardial protection to identify disease biomarkers, to predict severe morbidity, and thus to provide preventive, diagnostic, therapeutic, and evaluation criteria, and to further assist in the development of an exciting therapeutic strategy, may provide more meaningful treatment options for heart diseases caused by myocardial I/R injury in elderly patients. Our current study is aimed at several of the aforementioned points. These results may facilitate the extensive clinical utility of the relevant models, and warrant broadening the scope of the therapeutic blockade of expression of XIAP in myocardial ischemia and reperfusion injury in the heart. However, those in the XIAP transgenic mice appear insufficient to sufficiently detect these cases. That is why, theoretically, aging causes the heart to change at the gene sequence level, but subtle changes can be found in the gene sequence level, albeit with changes at the gene sequence level that occur which are adequate to achieve disease status. The subsequent questions will need to be addressed in future work. This work is of course ongoing and includes analyses to date, and in the absence of absolute proof of the role of XIAP in age-dependent isoform expression, this work aims to draw meaningful conclusions from these findings.

Future Directions

While the present study delineates the potential role of XIAP in attenuating myocardial injury in aging hearts after I/R, PI3K/AKT/MMP-2 activation downstream of XIAP in modulating myocardial protection has not been investigated. Additionally, whether XIAP overexpression in young or elderly hearts affects the maladaptive hypertrophic response is crucial to be further characterized. The determination of the role of XIAP that attenuates myocardial injury in aging hearts in clinical surroundings and the development of a more stable and effective method to retrieve protein XIAP function make reperfusion therapy, especially for patients with aging or diabetes-related MI, more successful.

Thinking about the age-related impact on MI, optimization of reperfusion strategy, and development of new therapeutic targets would be priorities for experimental and clinical cardiologists. This study highlights that proper XIAP treatment improves the outcomes of young and aged hearts after acute MI through regulating different cell signaling in both age hearts.

Research Article doi: 10.18081/ajbm/2333-5106/014-04/400-421

Therefore, XIAP may become a potential treatment target to treat the age-related cardiac damages after MI. It is particularly important to evaluate impacts in each experimental model at different age stages and select the appropriate path for efficient cardioprotection at different stages.

Conclusion

Our results revealed that XIAP is downregulated in the aged myocardium, which increases myocardial infarction caused by MIR and initiates apoptosis in ischemic aged mouse hearts. Underlying this, the present study supports the key concept that the decrease in XIAP expression in aged hearts aggravates MI/R injury, and increasing XIAP expression using gene therapy attenuates MI/R injury in aged hearts. Thus, we provide a rationale for the importance of XIAP in regulating ageing-associated heart injury in clinical practice, which could contribute to the reduction of MI/R injury during cardiac surgery in aged patients.

In summary, XIAP expression is decreased in aged myocardium, and myocardial infarction caused by MIR is increased, initiating apoptosis in ischemic aged mouse hearts. XIAP overexpression enhances the resistance to myocardial ischemia and reperfusion injury and induces apoptosis in aged mice, which is closely related to the inhibition of the mitochondrial apoptosis pathway. These data may provide a rationale for the importance of XIAP in regulating aging-associated heart injury in clinical practice, with potential implications for the reduction of MI/R injury during cardiac surgery in elderly patients.

Funding

No funding was received.

Competing interests

The authors declare no conflict of interest.

Ethics Statement

Not applicable.

Authors' contributions

All authors shared in the conception and design and interpretation of data, drafting of the manuscript and critical revision of the case study for intellectual content and final approval of the version to be published. All authors read and approved the final manuscript.

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Research Article doi: 10.18081/ajbm/2333-5106/014-04/400-421

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American Journal of BioMedicine
Journal Abbreviation: AJBM
ISSN: 2333-5106 (Online)
DOI: 10.18081/issn.2333-5106
Publisher: BM-Publisher
Email: editor@ajbm.net