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Aging might increase myocardial ischemia / reperfusion-induced apoptosis in humans and rats

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Abstract Previous studies indicated aging results in the significant cardiac function decreasing and myocardial apoptosis increasing in normal humans or rats. Additionally, animal experiments demonstrated aging increased myocardial ischemia / reperfusion (MI/R)-induced apoptosis. However, whether more myocardial apoptosis happen in the old acute myocardial infarction (AMI) patients is unclear. Reperfusion injury-induced apoptosis is an important cause of heart failure. This study determined the effect of aging upon myocardial apoptosis and cardiac function in patients suffering AMI. All enrolled AMI patients received percutaneous coronary intervention therapy. Volunteers and AMI patients were assigned to four groups: adult (age <65, n=24) volunteers, elderly (age ≥65, n=21) volunteers, adult (age <65, n=29) AMI patients, and elderly (age ≥65, n=36) AMI patients. Blood samples were obtained from all study participants. Plasma apoptotic markers (soluble form of Fas, tumor necrosis factor alpha, and interleukin 6) levels were determined. Cardiac function was evaluated with echocardiogram and Killip class. Due to lack of a direct apoptotic assay method in live human subjects, an additional animal experiment was performed. Both young (2 months) and old (24 months) rats were subjected to 30-min myocardial ischemia and 3 (for TUNEL/caspase activity apoptotic assay) or 24-h (for cardiac function determination) reperfusion. Compared to adult patients, the elderly patients manifested decreased cardiac function and increased plasma apoptotic marker levels significantly. The animal experiment results (cardiac function and plasma apoptotic markers assays) were consistent with the human result data. Animal TUNEL staining and caspase activity measurement revealed a higher myocardial apoptotic ratio in the older rat group. Aging exacerbated MI/R injury in humans and rats. Differential myocardial apoptosis may play a vital role in mediating the observed effects.

Keywords Aging · Acute myocardial infarction (AMI) · Apoptosis · Percutaneous coronary intervention (PCI)

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Introduction

The rapid growth of the world elderly population has heightened awareness of age-related diseases, including interest in the study of the aging human heart. By 2020, those older than 60 years will account for more than 20% of the total population, corresponding to the fastest growing age group worldwide. Cardiovascular diseases are the leading causes of death in the elderly, as those older than 65 years account for greater than 80% of patients with ischemic heart disease (Rosamond et al. 2007). Additionally, the exponential increase in mortality rate related to cardiovascular diseases in the geriatric population implies that cardiac aging itself may be a major risk factor for cardiovascular pathology such as ischemic heart disease.

The growing evidence from both animal experiments and clinical observations indicates that myocardial infarction after ischemia and reperfusion is caused not only by necrosis, a traditional cell death pathway, but also by apoptosis, a gene-controlled programmed cell death (Gottlieb and Engler 1999). Data from Kajstura et al. demonstrated that apoptosis in the left ventricles increased by more than 200% in 24-month-old rats compared with 16-month-old rats, but there was no quantitative change in necrotic cell death, strongly suggesting that apoptosis may be more prevalent than necrosis in old rats (Kajstura et al. 1996). Phaneuf and Leeuwenburgh demonstrated that Bax, Bcl-2, and cytochrome *c* play important roles in aging-related myocardial apoptosis (Phaneuf and Leeuwenburgh 2002). Although the relationships between heart failure and apoptosis (Olivetti et al. 1997), aging and heart failure had been confirmed, but seldom do research involves the effects of aging on apoptosis in humans.

Although several animal studies have demonstrated significantly more apoptosis prevalent in the aged heart (Shih et al. 2011; Dai and Rabinovitch 2009), two questions remain unanswered. Firstly, what is the effect of aging upon ischemia-induced apoptosis? Secondly, and more importantly, what are the effects of aging upon human myocardial apoptosis?

The major aim of the present study was to investigate the effects of aging upon post-ischemia myocardial apoptosis in humans utilizing indirect plasma apoptotic markers. Due to experimental limitations with a clinical trial, an additional animal experiment evaluated apoptotic index via TUNEL staining and caspase-3 activity determination, which were used in the present study.

Materials and methods

The clinical trial was carried out in accordance with the Declaration of Helsinki of the World Medical Association (2000). The study protocol was approved by the institutional ethics committee in Beijing Chaoyang Hospital—Affiliate of Beijing Capital Medical University. After being given full explanation of the study's purpose, nature, and any inherent risk involved with the participation, all subjects gave written informed consent prior to study inclusion.

Inclusion and exclusion criteria of acute myocardial infarction patients

Healthy volunteers and patients with acute myocardial infarction (AMI) were included in this study. Inclusion criteria for healthy volunteers were: (1) absence of chest pain or shortness of breath; (2) normal electrocardiograph, without significant alteration in Q waves, T waves, or S-T segment morphology; (3) absence of heart disease history; (4) absence of inflammatory or autoimmune disease history; and (5) absence of significant other comorbidities. AMI patients met inclusion criteria if two or more of the following conditions were true: (1) presence of chest pain, (2) presence of electrocardiographic changes with Q waves (representative of transmural infarction), or (3) elevated serum creatinine kinase levels. Nontransmural infarction was diagnosed by S-T segment or T-wave changes accompanied by increased creatine kinase–myoglobin fraction concentrations. Exclusion criteria for this study included: (1) cardiogenic shock, (2) left main coronary artery occlusion or severe stenosis, (3) blood flow in the infarct-related artery $\geq$ thrombolysis in myocardial infarction grade 1, (4) obvious coronary collaterals to the risk region evidenced by Rentrop grade $\geq$1, (5) previous myocardial infarction, (6) treatment with glycoprotein IIb/IIIa receptor antagonists prior to catheterization, and (7) major infection or surgery within the past 2 weeks prior to presentation.

Coronary angiography and clinical experimental design

All AMI patients were examined at admission and pre-medicated with clopidogrel (300–600 mg) and aspirin (300 mg) before catheterization. Clinical percutaneous coronary intervention (PCI) was completed as described (Fan et al. 2010). After PCI, all patients received the treatments according to guidelines (King et al. 2008; Campbell-Scherer and Green 2009).

The healthy volunteers were divided into two groups: (1) adult volunteers (<65 years) and (2) old volunteers (≥65 years). AMI patients were divided into two groups: (1) adult AMI patients (<65 years) and (2) old AMI patients (≥65 years).

Killip class was assessed independently by two cardiologists at admission and 5 days after PCI. Left ventricular ejection fraction (LVEF), left ventricular fractional shortening (LVFS), and E/A values were determined via echocardiogram at admission and 5 days after PCI. Blood samples were drawn for analyses at 24 h and 3 and 5 days after PCI. The healthy volunteers were subject to cardiac function index and blood draw similarly.

Animal experiment protocol

The study was approved by the institutional ethics committee and confirmed with the United States National Institutes of Health guidelines. Male Sprague–Dawley rats were anesthetized with sodium pentobarbital. Myocardial ischemia was produced by exteriorizing the heart via a left thoracic incision and occluding the left coronary artery (LCA) with a silk slipknot. After 30 min of ischemia, the slipknot was released and the myocardium was reperfused for 3/24 h. The sham-operated control rats (sham) underwent the same surgical procedures except that the LCA was not occluded.

Male Sprague–Dawley rats (8 weeks and 24 months) were randomized to receive either sham or myocardial ischemia (MI) and were divided into the following groups (n=8 each): (1) young sham, (2) old sham, (3) young MI, and (4) old MI.

Determination of myocardial functional recovery in rats

Left ventricular (LV) function was continuously monitored via a Millar Mikro-Tip catheter pressure transducer inserted into the LV via left carotid artery as previously described (Gao et al. 2010). Left ventricular end diastolic pressure (LVDEP), left ventricular systolic pressure (LVSP), and rate of rise of left ventricular pressure (±dp/dt) were derived by computer algorithms (Chengdu Instrument Co., Chengdu, China).

Determination of myocardial infarct size in rats and humans

After 24-h reperfusion, the infarct size in rat hearts was measured with a double-staining technique using Evans blue–triphenyltetrazolium chloride staining, and a digital imaging system, as previously described (Fan et al. 2005; An additional eight rats per group were dedicated for infarct size determination). Blood samples were obtained at the conclusion of 24-h reperfusion.

Plasma cardiac specific troponin I (cTnI) from rats and patients (24 h and 3 and 5 days after PCI) were measured spectrophotometrically (Beckman DU 640, CA) with commercially available assay kits (Nanjing Jiancheng Co., Nanjing, China). The plasma samples were coded, and cTnI levels were determined in duplicate by an investigator blinded to the research groups.

Determination of rat myocardial apoptosis and caspase-3 activity

Myocardial apoptosis was determined by the combination of histochemical staining (TUNEL staining) and caspase-3 activity detection. Immunohistochemical staining was employed to detect apoptotic cardiomyocytes via an in situ apoptosis detection kit (Roche Co., Basle, Switzerland) per manufacturer's instructions.

Caspase-3 activity (the final common pathway in caspase-dependent apoptosis) was determined in ischemic rat heart tissue by using a caspase-3 colorimetric assay kit per manufacturer's instructions (Chemicon International, Temecula, CA, USA) as previously reported (Fan et al. 2010). The samples were incubated at 37°C for 1.5 h, and the p-nitroanilide production was measured at 405 nm. The results were presented as the fold increase in caspase-3 activity relative to that of the MI group.

Determination of plasma apoptotic markers (soluble form of Fas, tumor necrosis factor alpha, and interleukin 6) in control groups and AMI patients

The blood samples were drawn from healthy control and AMI patients after 24 h and 3 and 5 days

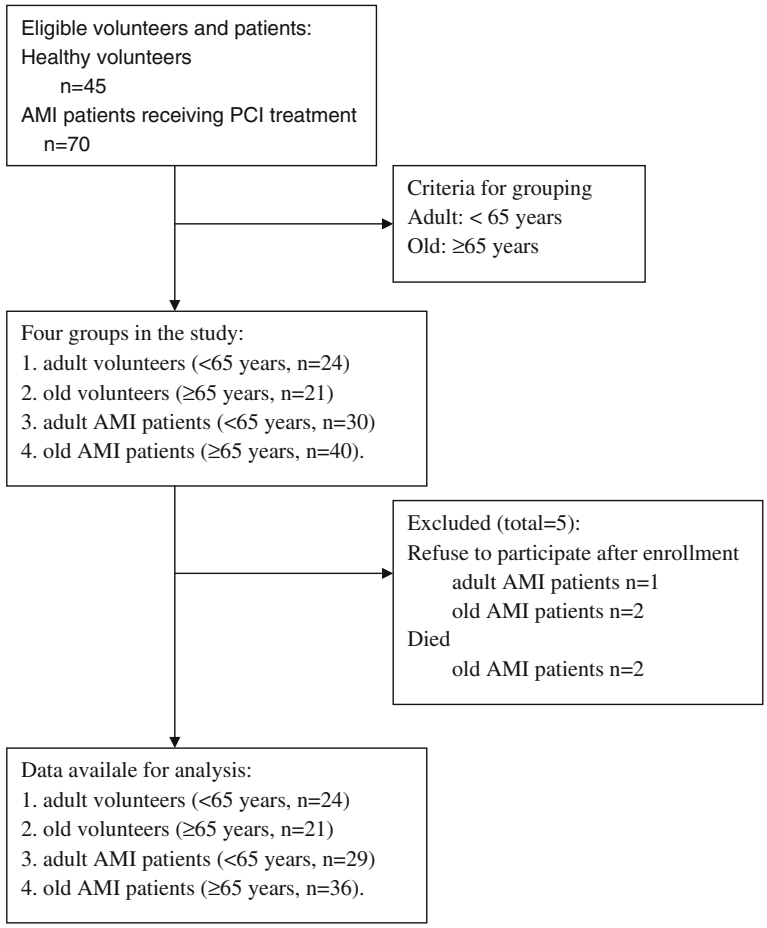


Fig. 1 The grouping of clinical trial

reperfusion. The blood samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C, and the supernatant was collected and stored at −80°C until measurement. Fas and Fas ligand (FasL) are cell surface proteins and both have a soluble form. Fas induces apoptosis when it binds to FasL or to the soluble form of FasL. Tumor necrosis factor alpha (TNF-α) induces apoptosis through the binding of its specific cell membrane receptor, TNF-R1, triggering the extrinsic pathway. Interleukin 6 (IL-6) is an inducer of apoptosis related to the Fas/FasL system, which induces the transcription of the Fas/APO 1 receptor gene. Therefore, in the present study, we used soluble form of Fas (sFas), TNF-α, and IL-6 as apoptotic markers (Cristobal et al. 2010). Plasma sFas, TNF-α, and IL-6 concentration were detected with commercially available kits (Human soluble factor-related apoptosis, sFAS/Apo-1 ELISA Kit, Wuhan EIAab Science Co., Ltd, China; Human TNF-α ELISA Kit, Abcam, UK; Human IL-6 ELISA Kit, Abazyme LLC, USA).

Statistical analysis

All values are presented as means±SD. All biochemical assays were performed in duplicate and averaged. Data were subjected to ANOVA, followed by Bonferroni correction for post hoc Student's *t* tests. All statistics were calculated using Graphpad Prism 5.0. *P* values <0.05 were considered statistically significant.

Results

Basic characteristics in human study

As shown in Fig. 1, 24 adult healthy volunteers, 21 old healthy volunteers, 30 adult AMI patients, and 40

Table 1 Baseline characteristics of the study population (mean±SD)

	Health adult (n=24)	Health elderly (n=21)	P	MI adult (n=29)	MI elderly (n=36)	P
Age, years	50.17±7.15	73.10±4.98	<0.01	54.90±5.14	75.69±6.86	<0.01
Sex, M/F	16/8	13/8	NS	16/13	20/16	NS
HBP/total	6/24	8/21	NS	18/29	32/36	<0.05
Dyslipidemia/total	6/24	9/21	NS	19/29	30/36	NS
Diabetes/total	3/24	7/21	NS	11/29	24/36	<0.05
Smoker/total	7/24	8/21	NS	13/29	15/36	NS
Culprit Arteries number				1.31±0.60	2.78±0.48	<0.01
Past drug treatment (n/total)						
Statins	4/24	9/21	NS	12/29	30/36	<0.01
Calcium channel blocker	0/24	1/21	NS	4/29	8/36	<0.01
ACEI	3/24	4/21	NS	8/29	21/36	<0.05
ß-Adrenoceptor blocker	4/24	7/21	NS	9/29	20/36	NS
Drug treatment during the study (n/total)						
Statins	4/24	9/21	NS	19/29	30/36	NS
Calcium channel blocker	0/24	1/21	NS	7/29	9/36	NS
ACEI	3/24	4/21	NS	27/29	32/36	NS
ß-Adrenoceptor blocker	4/24	7/21	NS	23/29	31/36	NS

HBP high blood pressure, *ACEI* angiotensin-converting enzyme inhibitor, *M* male, *F* female

old AMI patients were enrolled in the clinical trial. Primary PTCA with stent placement was successful in all patients. Of 70 AMI patients, one adult and two old patients refused study participation after enrollment, and two old patients died after 5 days in the study. All healthy volunteers completed the study. Demographic data, baseline statistics, cardiovascular risk profile, medication profile, and clinical measures of cardiac function of all enrolled subjects are shown in Table 1. The major difference between adult and old patient groups is that the latter had significantly more infarction-related arteries.

Aging depressed cardiac function in humans and rats

Clinical trial

Consistent with previous studies, as shown in Fig. 1, cardiac systolic function (LVEF and LVFS) and diastolic function (E/A ratio) in adult volunteers were significantly improved compared to the elderly. In regard to post-ischemic cardiac function, LVEF (Fig. 2d), LVFS (Fig. 2e), and E/A ratio (Fig. 2f) were significantly greater in the adult group than old AMI group at admission and day 5 after PCI. Consistent with these data, Killip class value of the adult group was lower than that in old group (Fig. 2g).

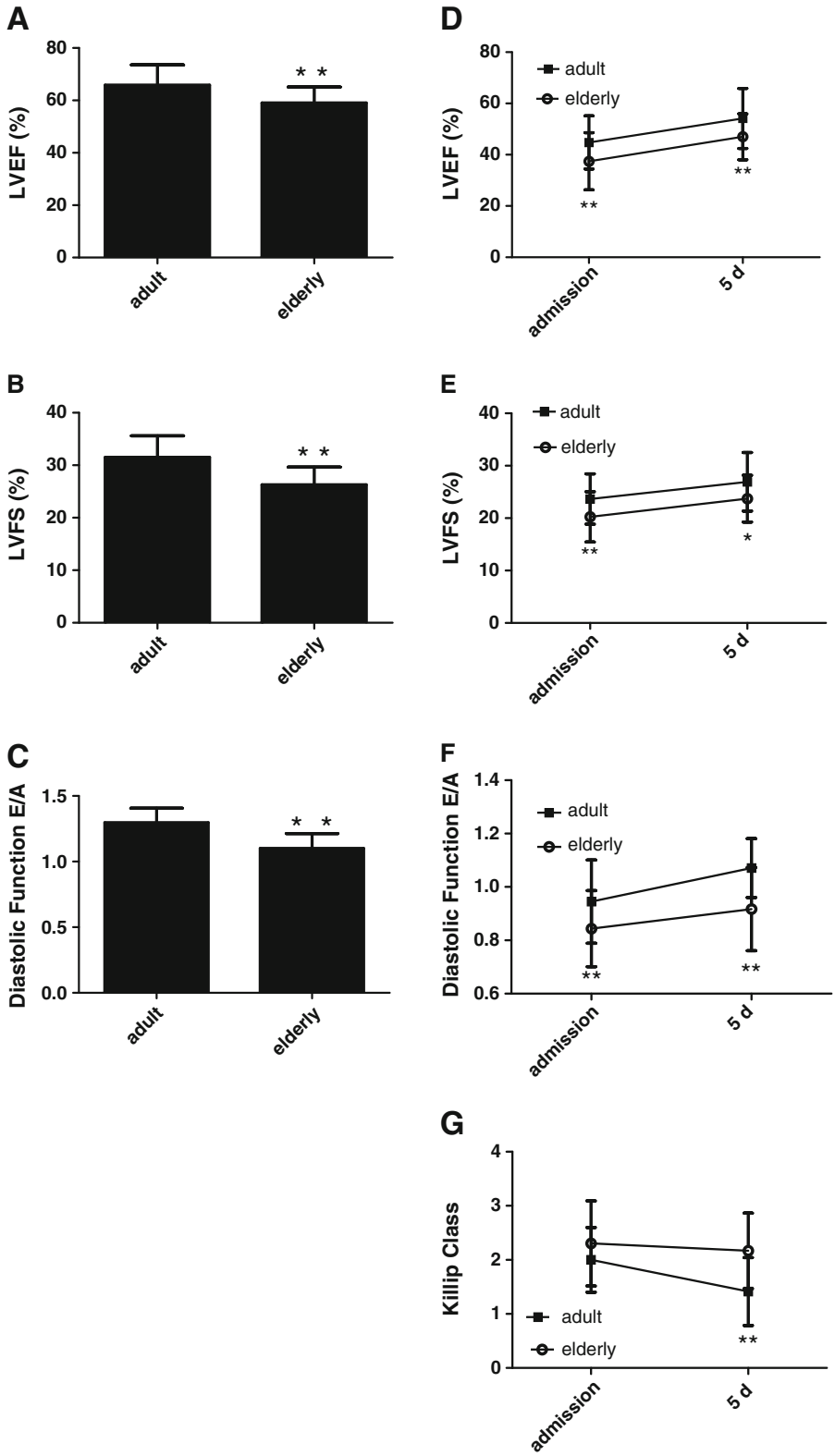
Animal experiment

Rat experimental results reinforced the human data findings. Utilizing a Millar Mikro-Tip catheter and pressure transducer, direct cardiac function index, LVSP, LVEDP, and ±dp/dt values were obtained. In non-ischemic conditions, older rats manifested decreased LVSP, decreased ±dp/dt, and greater LVEDP in comparison to younger rats. MI/reperfusion (R) injury significantly decreased LVSP and ±dp/dt and increased LVEDP in all groups. In the two groups of ischemic rats, LVSP and ±dp/dt were lower, while LVEDP was higher significantly in older group (Fig. 3).

Aging exacerbated ischemia / reperfusion-induced myocardial necrosis

Clinical trial

To determine the effects of aging upon post-ischemic myocardial necrosis, we detected the plasma levels of cTnI, a marker reflecting myocardial cellular damage at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◀ **Fig. 2** Aging associated with decreased cardiac function in healthy volunteers and AMI patients. **a** Left ventricular ejection fraction (LVEF), **b** left ventricular fractional shortening (LVFS), and **c** E/A value depict volunteer data. **d** LVEF, **e** LVFS, **f** E/A value, and **g** Killip class depict AMI data. *Adult* indicates adult group; *elderly* indicates the elderly group. Total for the following groups: adult volunteers—24, old volunteers—21, adult patients—29, and old patients—36. Data expressed as mean±SD. *$P<0.05$, **$P<0.01$ vs. adult group

three distinct time points (24 h and 3 and 5 days after PCI). Plasma cTnI concentration in old AMI patients was elevated in comparison to the younger group (Fig. 4c).

Animal experiment

In addition, we detected the area of infarction (white)/area at risk (red) ratio in rat MI/R model. There were not significant necrotic zone in non-ischemic rat. However, 30-min ischemia and 24-h reperfusion resulted in a significant myocardial necrosis. Compared with young MI group, the ratio was significantly higher in old MI group. The results were consistent with cTnI measurement.

The results of the clinical study were consistent with the animal study. As shown in Fig. 4a, b, aging exaggerated myocardial infarct size compared to the younger MI group, accompanied with increased plasma cTnI levels.

Aging increased ischemia / reperfusion-induced myocardial apoptosis in rats

Apoptotic extent was assessed via TUNEL staining and caspase-3 activity. In non-ischemic rat hearts, aging resulted in a slight, but significant, increase in TUNEL-positive cells and caspase-3 activity (Fig. 5). Thirty minutes of MI followed by 3 h of R resulted in significant myocardial apoptosis, as evidenced by a marked increase in TUNEL-positive cells and caspase-3 activation. Compared to young rats, ischemia (I)/R) resulted in greater myocardial apoptotic ratio and caspase-3 activity in old rat cardiac tissue.

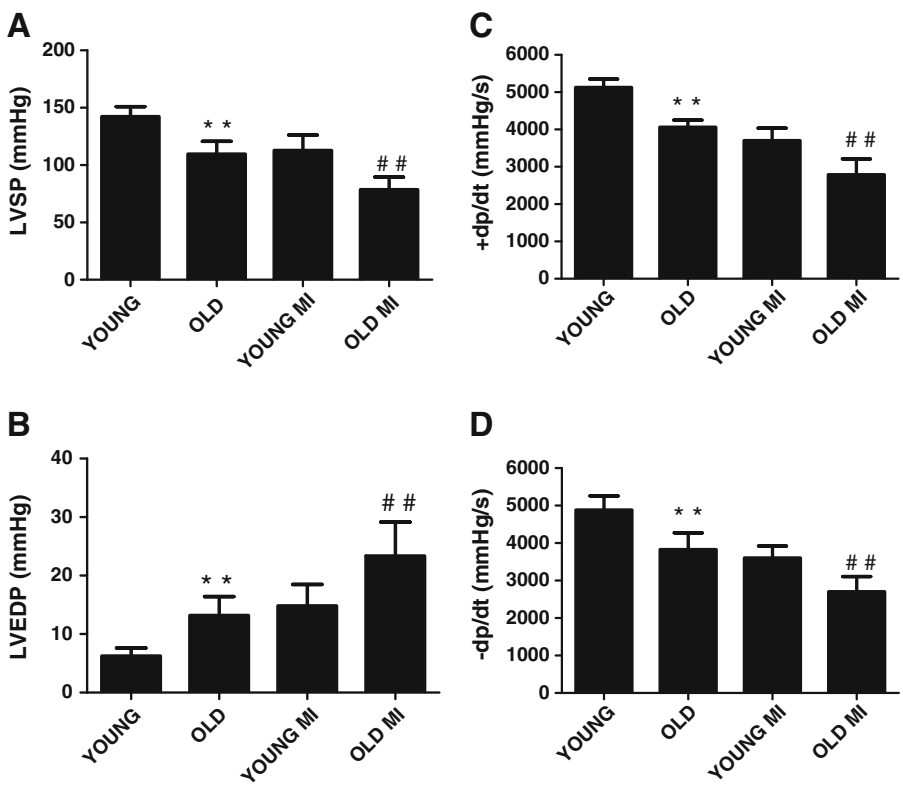

Fig. 3 Aging associated with decreased cardiac function in rats after 24-h reperfusion. **a** Effects of aging upon left ventricular systolic pressure (LVSP). **b** Left ventricular end diastolic pressure (LVDEP). **c**, **d** Rate of rise of left ventricular pressure (±dp/dt). *Young* indicates young sham group; *old* indicates old sham group; *young MI* indicates young I/R group; *old MI* indicates old I/R group. $n=8$ in each group. Data expressed as mean±SD. *$P<0.05$, **$P<0.01$ vs. adult group, $^{\#\#}P<0.01$ vs. young MI group

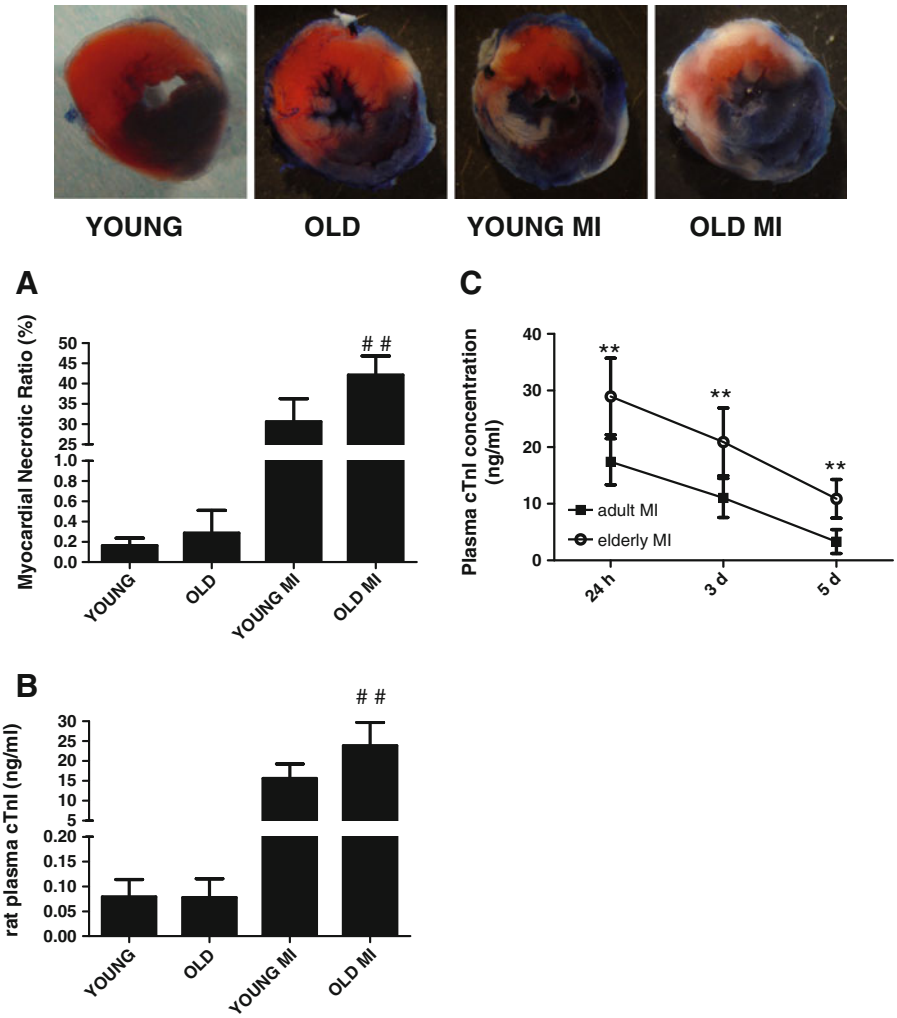

Fig. 4 Effects of aging upon MI/R injury in humans and rats. *Top* Representative photomicrographs of Evans blue–triphenyltetrazolium chloride (TTC) staining in sectioned rat heart tissue. *Bottom* **a** Bar chart of myocardial infarct size (IS) expressed as percentage of area at risk (AAR) of the ischemic-reperfused myocardium. **b** Plasma cTnI concentration in different rat groups. **c** Plasma cTnI concentration in AMI patients 24 h, 3 days, and 5 days after PCI. Data were expressed as mean±SD. *$P<0.05$, **$P<0.01$ vs. young/adult group, $^{\#\#}P<0.01$ vs. young MI group

Aging associated with increased plasma apoptotic marker levels in humans and rats

Clinical trial

Our TUNEL staining and caspase-3 activity assays provided direct evidence that aging increased myocardial apoptosis in rats after I/R. We next determined whether similar effects of aging were present in humans. We assessed the levels of plasma apoptotic markers (sFas, TNF-α, and IL-6) in AMI patients, and found increased levels in the elderly compared to the younger group after 24 h and 3 and 5 days after PCI (Fig. 6). However, a significant difference in the number of culprit arteries existed between the adult and the old patient groups. Although the data supported aging increased post-ischemic myocardial apoptosis, it is uncertain whether more severe ischemic episodes were responsible for the obtained data.

Animal experiment

To answer this question, we tested plasma apoptotic markers in rats. The results indicated MI/R caused a marked increase in plasma sFas, TNF-α, and IL-6 level (Fig. 7). Furthermore, plasma apoptotic marker levels in old ischemic rats were significantly greater than those of the young group, in consistent fashion with our clinical trial data.

Discussion

Although the risk of mortality from acute MI and coronary artery disease has declined with available

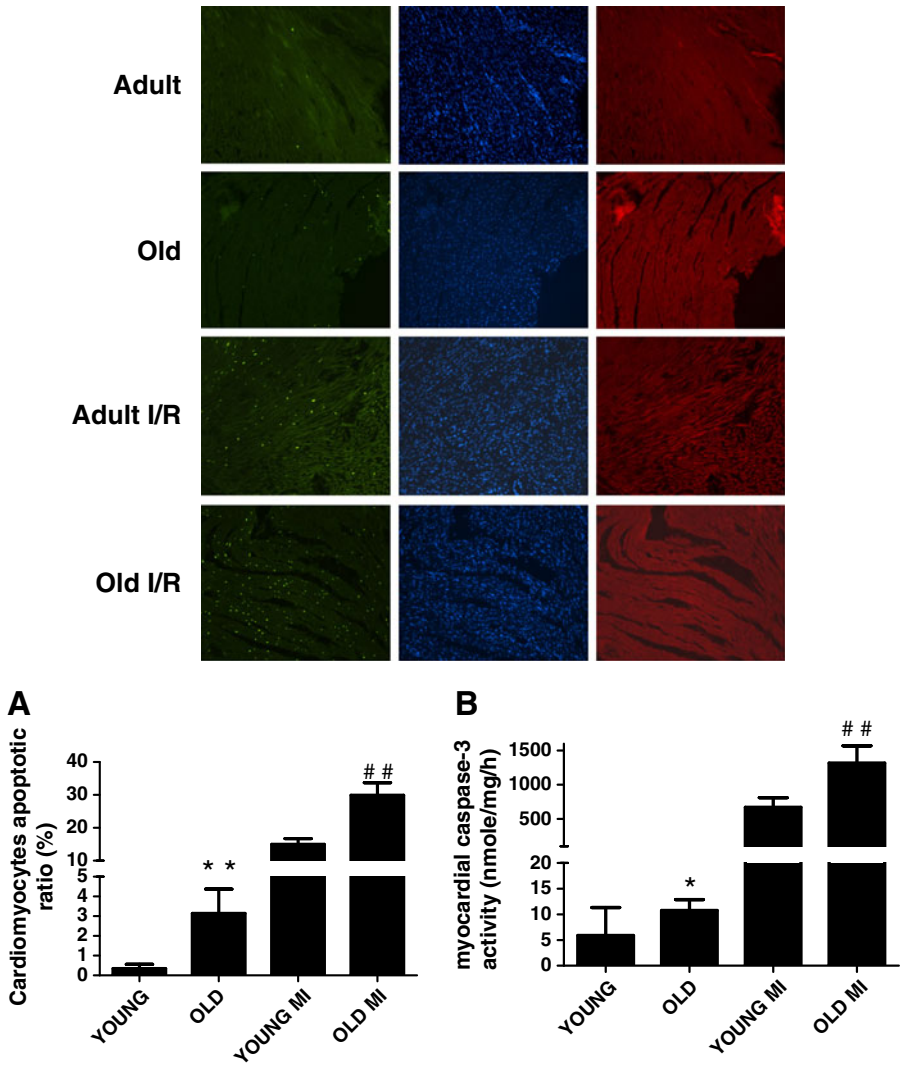

Fig. 5 Effects of aging upon I/R-induced myocardial apoptosis in rats. *Top* Representative photomicrographs of TUNEL staining in all four groups. Total nuclei were labeled with DAPI (*blue*) and apoptotic nuclei appear *green* by TUNEL staining. **a** TUNEL-positive myocytes. **b** Caspase-3 activity. $n=8$ in each group. Data expressed as mean± SD. $^{*}P<0.05$, $^{**}P<0.01$ vs. young group, $^{\#\#}P<0.01$ vs. young MI group

coronary interventions, the number of hospitalizations of post-ischemia heart failure as either the principal or the secondary diagnosis in patients over the age of 65 has actually increased by 70% to 100% over the last quarter century (Fang et al. 2008). Therefore, a key focus of the treatment of AMI has shifted from reperfusion to retardation of heart failure development. However, what is the major cause of post-ischemia heart failure? One of our previous studies demonstrated apoptosis exerts a vital effect in mediating the development of post-ischemia heart failure. Furthermore, it is possible that apoptosis is the major cause of post-ischemia heart failure.

Previous studies have shown also that advanced age is a major risk factor for heart failure development (Shih et al. 2011; Curtis et al. 2008). Additionally, numerous reports have demonstrated that apoptosis plays a vital role in MI/R and post-ischemic heart failure pathogenesis (Narula et al. 1996; Das 2007; Hikoso et al. 2007; Dai and Rabinovitch 2009). Heart failure incidence increases sharply with age. What causes age-associated heart failure, and what is its relation to post-ischemic heart failure? To date, the precise mechanisms have remained unclear. Furthermore, experiments performed on adult animals (Zhang et al. 2007a, b) have indicated the important role that apoptosis plays in post-ischemic heart failure, but the effect of aging upon ischemia-related apoptosis in humans is unknown.

Several important observations were made in the present study: (1) aging increased the plasma apopto-

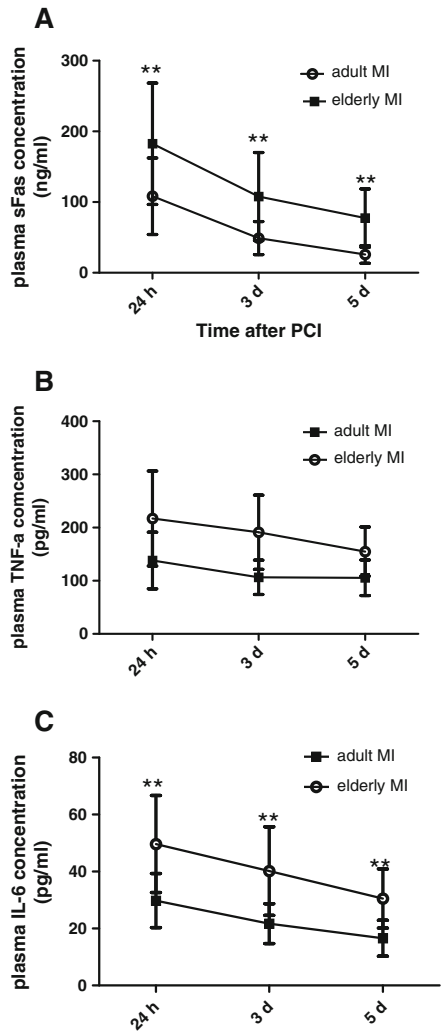

Fig. 6 Aging increased plasma apoptotic markers (sFas, TNF-α, and IL-6) in patients 24 h, 3 days, and 5 days after PCI. Patient plasma **a** sFas, **b** TNF-α, and **c** IL-6 concentrations. Total for the following groups: adult volunteers—24, elderly volunteers—21, adult patients—29, and elderly patients—36. Data expressed as mean±SD. *$P<0.05$, **$P<0.01$ vs. adult group

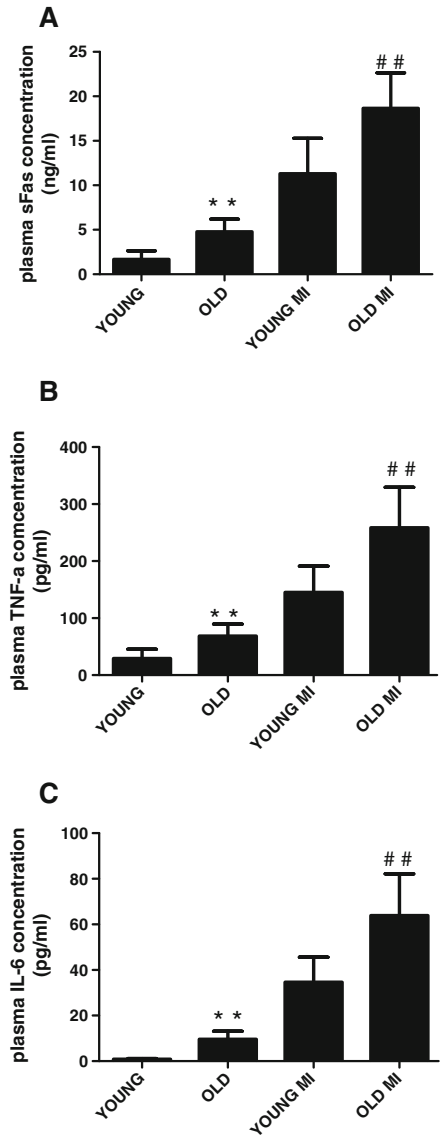

Fig. 7 Aging increased plasma apoptotic markers (sFas, TNF-α, and IL-6) in rats after 24 h I/R. Rat plasma **a** sFas, **b** TNF-α, and **c** IL-6 concentrations. $n=8$ in each group. Data expressed as mean±SD. *$P<0.05$, **$P<0.01$ vs. young group, $^{\#\#}P<0.01$ vs. young MI group

tic markers concentration in AMI patients after PCI and (2) aging decreased cardiac functional recovery after PCI. Animal data confirmed the clinical data of this study, with repeated, direct measures of apoptosis. To our knowledge, the present report is the first study of the relationship between aging and ischemia-related myocardial apoptosis in AMI patients.

Currently, there is no available direct measurement of human myocardial apoptosis with plasma. We utilized sFas, TNF-α, and IL-6 as apoptotic markers to measure cardiomyocyte apoptotic level in AMI patients. The Fas/FasL system is a vital mediator of myocardial apoptosis. The sFas is produced by alternative splicing of the transcript and circulates in the blood. Increased serum sFas concentrations are considered to reflect Fas/FasL system activation in vivo. TNF-α and IL-6 are the proinflammatory cytokines with crucial roles in cardiomyocyte apoptosis. Furthermore, the serum concentrations of sFas, TNF-α, and IL-6, which have been reported as

reliable cytosolic markers of apoptosis (Trikas et al. 2005; Ramalingam et al. 2008; Kavathia et al. 2009; Lanfear et al. 2009; Niessner et al. 2009), correlate with heart disease severity.

However, sFas, TNF-α, and IL-6 are not direct indices evaluative of in vivo apoptotic ratio. Animal studies were therefore utilized to obtain cardiac tissue, and directly measure myocardial apoptotic cell ratio. Our animal study results are consistent with those done previously. In 2002, Liu et al. reported age aggravated MI/R injury via gene expression changes in Bax and Bcl-2, ultimately increasing the ischemia / reperfusion-induced myocardial apoptotic ratio (Liu et al. 2002). Subsequent studies supported their findings and revealed the involvement of multiple factors, including cytochrome *c*, reactive oxygen species, connective tissue growth factor, calcineurin, serine protease, and apoptosis inducing factor. Both others' work and our current study demonstrated the close relationship between age and myocardial apoptosis. Furthermore, recent evidence indicates myocardial apoptosis might be a major cause of post-ischemic heart failure development (Guo et al. 2008). Our study not only demonstrated age-induced exacerbation of myocardial apoptosis in animals, but in humans as well.

In summary, our clinical trial demonstrated there was a correlation between aging and myocardial apoptosis; furthermore, the animal study provided evidences to confirm this correlation. Combined with recent evidence that apoptosis might be the major cause of heart failure, our data support the vital role of apoptosis in heart failure of the elderly population.

Limitation

As shown in Table 1, there were significant differences in several indexes (HBP, diabetes, culprit arteries number, and past drug treatment) between adult and old AMI patients. Especially, the number of culprit arteries is a direct and major factor on the ischemia-induced necrosis and apoptosis. The number of culprit arteries in the adult AMI group was significantly less than the old AMI group. The measurement of myocardial necrotic markers indicated more severe myocardial ischemia in the elderly. Due to natural limitations inherent of clinical trials, it is difficult to enroll exactly similar patient cases harboring the same number of PCI-determined culprit arteries. During our study's animal experiments, the same artery was blocked in the same location in each animal tested to compensate for the clinical trial limitations.

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References

Campbell-Scherer DL, Green LA (2009) ACC/AHA guideline update for the management of ST-segment elevation myocardial infarction. Am Fam Physician 79(12):1080–1086

Cristobal C, Segovia J et al (2010) Apoptosis and acute cellular rejection in human heart transplants. Rev Esp Cardiol 63 (9):1061–1069

Curtis LH, Whellan DJ et al (2008) Incidence and prevalence of heart failure in elderly persons, 1994–2003. Arch Intern Med 168(4):418–424

Dai DF, Rabinovitch PS (2009) Cardiac aging in mice and humans: the role of mitochondrial oxidative stress. Trends Cardiovasc Med 19(7):213–220

Das M (2007) Apoptosis as a therapeutic target in heart failure. Am J Physiol Heart Circ Physiol 293(3):H1322–H1323

Declaration of Helsinki of the World Medical Association (2000) Ethical principles for medical research involving human subjects. Jama 284(23):3043–3045

Fan Q, Gao F et al (2005) Nitrate tolerance aggravates postischemic myocardial apoptosis and impairs cardiac functional recovery after ischemia. Apoptosis 10(6):1235–1242

Fan Q, Yang XC et al (2010) Postconditioning attenuates myocardial injury by reducing nitro-oxidative stress in vivo in rats and in humans. Clin Sci (Lond) 120(6):251–261

Fang J, Mensah GA et al (2008) Heart failure-related hospitalization in the U.S., 1979 to 2004. J Am Coll Cardiol 52(6):428–434

Gao E, Lei YH et al (2010) A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. Circ Res 107(12):1445–1453

Gottlieb RA, Engler RL (1999) Apoptosis in myocardial ischemia–reperfusion. Ann NY Acad Sci 874:412–426

Guo Y, He J et al (2008) Locally overexpressing hepatocyte growth factor prevents post-ischemic heart failure by inhibition of apoptosis via calcineurin-mediated pathway and angiogenesis. Arch Med Res 39(2):179–188

Hikoso S, Ikeda Y et al (2007) Progression of heart failure was suppressed by inhibition of apoptosis signal-regulating kinase 1 via transcoronary gene transfer. J Am Coll Cardiol 50(5):453–462

Kajstura J, Cheng W et al (1996) Necrotic and apoptotic myocyte cell death in the aging heart of Fischer 344 rats. Am J Physiol 271(3 Pt 2):H1215–H1228

Kavathia N, Jain A et al (2009) Serum markers of apoptosis decrease with age and cancer stage. Aging Albany NY 1 (7):652–663

King SB 3rd, Smith SC Jr et al (2008) 2007 focused update of the ACC/AHA/SCAI 2005 Guideline Update for Percutaneous Coronary Intervention: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines: 2007 Writing Group to Review New Evidence and Update the ACC/AHA/SCAI 2005 Guideline Update for Percutaneous Coronary Intervention, Writing on Behalf of the 2005 Writing Committee. Circulation 117(2):261–295

Lanfear DE, Hasan R et al (2009) Short term effects of milrinone on biomarkers of necrosis, apoptosis, and inflammation in patients with severe heart failure. J Transl Med 7:67

Liu P, Xu B et al (2002) Age-related difference in myocardial function and inflammation in a rat model of myocardial ischemia–reperfusion. Cardiovasc Res 56(3):443–453

Narula J, Haider N et al (1996) Apoptosis in myocytes in end-stage heart failure. N Engl J Med 335(16):1182–1189

Niessner A, Hohensinner PJ et al (2009) Prognostic value of apoptosis markers in advanced heart failure patients. Eur Heart J 30(7):789–796

Olivetti G, Abbi R et al (1997) Apoptosis in the failing human heart. N Engl J Med 336(16):1131–1141

Phaneuf S, Leeuwenburgh C (2002) Cytochrome *c* release from mitochondria in the aging heart: a possible mechanism for apoptosis with age. Am J Physiol Regul Integr Comp Physiol 282(2):R423–R430

Ramalingam S, Kannangai R et al (2008) Investigation of apoptotic markers among human immunodeficiency virus (HIV-1) infected individuals. Indian J Med Res 128 (6):728–733

Rosamond W, Flegal K et al (2007) Heart disease and stroke statistics—2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 115(5):e69–e171

Shih H, Lee B et al (2011) The aging heart and post-infarction left ventricular remodeling. J Am Coll Cardiol 57(1):9–17

Trikas A, Papathanasiou S et al (2005) Left atrial function, cytokines and soluble apoptotic markers in mitral stenosis: effects of valvular replacement. Int J Cardiol 99(1):111–115

Zhang H, Tao L et al (2007a) Nitrative thioredoxin inactivation as a cause of enhanced myocardial ischemia/reperfusion injury in the aging heart. Free Radic Biol Med 43(1):39–47

Zhang XP, Vatner SF et al (2007b) Increased apoptosis and myocyte enlargement with decreased cardiac mass; distinctive features of the aging male, but not female, monkey heart. J Mol Cell Cardiol 43(4):487–491