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Acute Low-level Chronic Cigarette Smoke Exposure is always Harmful to Left Ventricular Function in older Rats

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Abstract

Background: Cigarette smoke (CS) exposure is associated with an increased risk of coronary artery disease. This study aim was to investigate the relationship of CS exposure in old left ventricle impaired. To explore the mechanism of cardiac remodeling of once exposure to CS exposure whether was exacerbated cardiac impaired, especially in old left ventricle. **Results:**Results showed LV wall and mass increased, collagen accumulation and fibrosis, and extracellular space increased in old rats in cigarette smoke exposure group (Old CSexp). From echocardiographic results, we found LV functions were apparently decreased, LV wall thickness and LV interventricular septum at systolic and diastolic diameters increased in Old CSexp group. Cardiomyocytes width was increased in old rats, but length was increased in Old CSexp rats. MMP-2 and MMP-9 proteins expression and pro-MMP2 activity reduced ECM degradation, but TIMPs were induced fibrosis increased. Calcineurin/NFAT, JNK1, p38, IL-6, TNFα were increased by western blotting and immunohitochemistry in Old CSexp. **Conclusions:**Cigarette smoke exposure induces cardiovascular disease resulted in left ventricular hypertrophy. However, old age leads the progressive accumulation of changes of the left ventricle. Inflammatory response accelerated CS exposure induced aging-related left ventricular hypertrophy. Therefore, we suggest that acute low-level chronic cigarette smoke exposure is also harmful to left Ventricular function in old rats.

Keyword: cigarette smoke exposure; left ventricle; cardiac impaired; left ventricular hypertrophy.

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Background

Cigarette smoke (CS) exposure is the risk of coronary heart disease. In the previous studies, CS exposure affected on the cardiovascular system, including atherosclerosis, increased arterial stiffness and coronary cardiac disease events [1]. CS exposure is the combination of smoke given off by the burred end of a tobacco or cigarette product to exposure to environment and the smoke exhaled by the smoker [2]. Most of CS is harmful and cause human diseases, especially in children [3,4]. However, low-level chronic cigarette smoke exposure harmful older adults are still unclear. In this study, we use 18-months-old rats underwent 15 cigarettes exposure 30 minutes to determined left ventricular remodeling and function. Hypertrophy is an initial adaptive response. There are much compensatory mechanisms to increase cardiac work-load and stimulation of left ventricular sustains [5]. However, aging is a progressive disease which is typic natural course whose the worsening of the disease until death occurs. Slowly progressive age-related diseases are also chronic diseases; many are also degenerative diseases [6]. However, a medical condition exists that is static disease. Age is the accumulation of changes in a person over time from birth. Aging changes that all people share is universal aging, but someone grow older including disease is probabilistic aging [7]. A mature organism that occur normally the gradual changes in the structure over time and increase the probability of death. This growing process is unavoidable. This physiologic changes of old cardiac includes left ventricular hypertrophy, increased cardiac fibrosis and valvular degeneration. Cardiovascular disease is a major risk factor for aging cause of death. Aging changes of the elderly heart is associated with physiological left ventricular hypertrophy (LVH). However, CS exposure is associated with pathological LVH [8]. CS exposure maybe leads to cardiovascular diseases such as heart failure and atherosclerosis. CS exposure in old heart is still unclear. Heart failure is a related changes in cardiac morphology, including decreased in myocyte number, increased in myocytes size [9], decreased in matrix connective tissue, increased in left ventricular wall thickness [10], increased in conduction fibre density and decreased in sinus node cell number [11]. CS exposure may stimuli first induce a phase of cardiac hypertrophy, especially in left ventricles individual [12]. Health aging changes may produce clinical heart disease and may mimic heart disease, such as cardiomyopathy, aortic valve calcium and mitral valve annular calcium [13]. Therefore, we detected the molecular mechanisms behind the aging in CS exposure treatment to identify pathological of cardiac disease disorder and elusive.

Methods

Animals

We purchased rats of 6 weeks years-old age from National Science Council Animal Center and used according to the guidelines of Helsinki Declaration. One group rats of 6-weeks-old rats as our young, another group rats of 18-months-old as our older age groups. Ten rats were housed in one cage in an environmentally controlled animal room. Use committee approved animal care and experiments. Animal room temperature is maintained at 25°C, and relative humidity was approximately 40%.

Cigarette smoke (CS) exposure

The old rats were placed in a whole-body transparent exposure chamber with a volume of approximately 95x85x85 cm, connected to a smoking device. Filtered air is introduced into the chamber at a low rate. Puffs of cigarette smoke were collected in the smoking chamber, being then thrown into the chamber for 30 minutes. The smoke is released at a rate of 15 cigarettes, twice a day in the morning and twice in the afternoon with 30 minutes rest intervals, until the end of 4 weeks.

Echocardiography

After 4 weeks of exposure treatment, all the animals underwent the echocardiographic study according to the previously described method. Animals used anesthetized with ketamine hydrochloride (50 mg/kg) and xylazine hydrochloride (1mg/kg). Transthoracic echocardiography was performed at 4 weeks after cigarette smoke (CS) exposure using a Hewlett-Packard Sonos 5500 ultrasound machine with a 7.5-15 MHz linear-array transducer, as described previously. In the short-axis parasternal view, we could obtain a transverse left ventricular one-dimensional image, the ultrasound beam right below the mitral valve plane between the papillary muscles by using the 2-dimensional image as a guide for positioning. M-mode image was recorded and analyzed offline. The following parameters were measured and calculated: left ventricular interior diastolic diameter (LVIDd), left ventricular interior systolic diameter (LVIDs), left ventricular interventricular septum at systolic and diastolic (LVIDs, LVIDd), left ventricular posterior wall thickness at systolic and diastolic (LVPWs, LVPWd), fractional shortening (FS%) and ejection fractional (EF%).

Hematoxylin - eosin (H&E) and Masson's Trichrome (MT) stained.

Left ventricular cross sections were cutted 10μm thick and placed on slides. Slides deparaffinization and dehydration were performed. They were passed through a series of graded alcohols from 100% to 90% to 70%, 5 min each. Hematoxylin - eosin and masson trichome stained were prepared, incubated for 5 min at room temperature. After rinsing with phosphate-buffered saline (PBS), each slide was then soaked with 85% alcohol, 100% alcohol for 5 min. After rinsing with water, each slide was then soaked with 85% alcohol, 100% alcohol for 15 min. Stained sections were then rinsed with PBS and air dried before mounting.

Gelatin Zymography

Proteins were separated by 8% non-reducing SDS-PAGE copolymerized with 1 mg/ml gelatin. The PAGE were washed at room temperature twice 10 minutes with 2.5% Triton-X 100, and subsequently incubated overnight at 37℃ for maximum sensitivity in Zymogram Developing Buffer mixture (50 mmol/L Tris-HCl, pH 7.4 containing 5 mmol/L CaCl2 and 1 μmol/L

ZnCl2). Gels were stained with Coomassie brilliant blue G250 (Methanol, Acetic acid and water mix) and then destained. The amounts of proenzyme and active metalloproteinase were analyzed by densitometry scanning of the gel.

Cell Size Distribution

Left ventricular cross-sections were performed H&E-stained. Color images of left ventricles cross-sections were made at 200x total magnification using a Nikon E600. Microscope lighting was optimized and increased the probability of cardiomyocytes being visualized in appropriate cross-sections and not-tangentially. Transverse axes and cross-sectional area were measured. Hypertrophic compensation or decompensation of the left ventricles can results in differences in the ratio of length or width to diameter of the cardiomyocytes, however, the volume of the cardiomyocytes was not assessed.

Tissue extraction

Left ventricular extracts were obtained by homogenizing the left ventricular samples in phosphate-buffered saline (PBS) at a concentration of 1 mg tissue/1 mL PBS for 5 min. The homogenates were placed on ice for 10 min and then centrifuged at 12,000 rpm for 30 min. The supernatant was collected and stored at –70°C for further experiments.

Protein contents

We determined the protein content of left ventricle tissue extract using the Bradford protein assay using the protein-dye kit. We used a commercially available bovine serum albumin as a standard. Changes in optical density were monitored at 595 nm.

Electrophoresis and Western blot

We prepared the tissue extract samples (40ug) as described above. SDS-PAGE was carried out with polyacrylamide gels. The samples were electrophoresed at 100 V for 1 hr. Electrophoresed proteins were transferred to PVDF membranes at 150 mA for 2 hr. We incubated PVDF membranes in blocking buffer (5% non-fat milk in PBS-Tween) for 1 hr at room temperature. Polyclonal antibodies against Calcineurin, NFAT, JNK1/2, p38α, IL-6, TNFα, MMP2, MMP9, TIMP-1, TIMP-2, TIMP-3 and TIMP-4 (Santa Cruz) were diluted 1:200 in antibody buffer (TBS). Incubations were performed at room temperature for 3.5 hr. We washed the immunoblots three times in 5 ml PBS-Tween for 10 min and then immersed in the second antibody solution containing alkaline phosphatase goat anti-rabbit IgG for 1 hr and diluted 1,000-fold in binding buffer. Color development was presented in ECL chemiluminescence.

Statistical analysis

Quantitation was carried out by scanning and analyzing the intensity of the hybridization signals using FUJIFILM Imagine program for western blot analysis. Statistical analysis of the data was performed using SigmaStat software. Results were expressed as mean ± SEM. Statistical analysis was performed using the analysis of variance. When assessing multiple groups, two-way ANOVA was utilized with turkeys post hoc test, unpaired, two-sided student's t test was used when indicated.

Results

Histopathologic of a left ventricular cross-sectional analysis assessed cardiac changes in old rats in cigarette smoke exposure by H&E stained and masson's stained.

To investigated the effects of cigarette smoke (CS) exposure on cardiac functions and structure changes were determined in rats model recommended for gerontological. Heart cross sections were stained with massons's trichrome or hematoxylin/eosin staining for visualization of morphology and identification of location. A cross-sectional analysis analysis assessed left ventriclular changes in old rats and old rats in CS exposure rats. As shown in Figure 1A. left ventricular chamber become narrowed in old rats in CS exposure group (Old CSexp). At the same time, CS exposure resulted in old rats left ventricular papillary muscle deteriorated which led to left ventricular dysfunction (Figure 1B). Left ventricular muscle fibers interstitial and extracellular space were broad (Figure 1C and 3B). Muscle fibers rearrangement is disordered. In old rats and old rats in CS exposure, we also could observe ECM degradation resulted in collagen release in cardiomyocytes interstitial (Figure 1D) and collagen accumulation induced fibrosis (Figure 1E and 1F). Indeed, from masson's trichrome stained results, we could observe blue color staining in cross- sections.

Changes in heart weight (HW), left ventricular weight (LVW), HW-to-body weight ratio, LVW-to-body weight ratio, HW-to-tibial ratio, and LV-to-tibial ratio in old rats and old rats in CS exposure.

Figure 2A, 2B and 2C. presents heart and left ventricular (LV) characteristics in young, old and old rats in CS exposure (Old CSexp). The whole heart weights of old rats and old rats in CS exposure were heavier than young. Aging and CS exposure were also enhanced the left ventricular weights (Figure 2A). HW-to-body weight and LV-to-body weight ratios were significantly increased compared with younger age group. Compared with old rats, HW-to-body weight and LV-to-body weight ratios also had significantly increased (Figure 2B) in Old CSexp groups. However, body weight is easily caused by the gradual increase in a consequence of aging. Sometimes affected by alcohol, smoke or toxic material effects. The use of tibia length has been evidenced more reliable than body weight. Because of in which body weight differences may occur conditions errors. Furthermore, we detected and measured HW-to-tibial and LV-to-tibial ratios to determine heart or left ventricular hypertrophy in old rats and old rats in CS exposure (Old CSexp). The result showed both of HW-to-tibial and LV-to-tibial ratios increased significantly as age increased, although exposure to CS exposure (Figure 3C). Therefore, we obtained HW-to-tibial and LV-to-tibial ratios increased compared with old rats groups.

Left ventricular function and structures development of left ventricle on echocardiographic analysis.

Echocardiographic analysis is a primary imaging method in the assessment of cardiac impaired and function declined (Figure 2D and 2E). Parasternal long-axis and short-axis echocardiographic views in young, old and old rats in CS exposure (Old CSexp) showing severe left ventricular hypertrophy. We found left ventricular wall thickness increased. However, from M-mode long-axis echocardiograms result taken proximal to the papillary muscle deterioration in old rats and old rats in CS exposure. On the other hand, we found left ventricular wall thickness increases in old rats and old rats in CS exposure at systolic and diastolic. Quantification of myocardial hypertrophy for old rats and old rats in CS exposure groups are displayed in Figure 3D. Echocardiography of interventricular septal in systolic (IVSs), interventricular septal in diastolic (IVSd), left ventricular internal dimension at end systolic (LVIDs), left ventricular internal dimension at end diastolic (LVIDd), left ventricular posterior wall thickness in systolic (LVPWs) and left ventricular posterior wall thickness in diastolic (LVPWd) were presented in young, old and old rats in CS exposure. The morphological variables obtained from the echoardiographic study are shown in Figure 2E. Old rats in CS exposure had greater IVSs, IVSd, LVIDs, LVIDd, LVPWs and LVPWd dimension compared with young rats. After exposure to CS, the CS exposure rats had statistically greater dimensions than nonsmoking old rats did. This variable change was used to confirm the efficacy of the exposure of old rats to cigarette smoke (CS). In addition, considering the left ventricular variables, the ejection and shortening fractions were significantly declined. As Figure 2E shown, shortening (FS%) and ejection fraction (EF%) displayed a progressive impairment in old rats and old rats in CS exposure.

Cardiomyocytes width and length of in left ventricular hypertrophy in old rats and old rats in CS exposure.

Phase of left ventricular hypertrophy during adaptive stress or overload is individual left ventricular myocyte grown in length and/or width as compensated or dilation hypertrophy. To detect whether CS exposure led left ventricular fibrosis exacerbated, we independently calculated the percentage of per cross-sectional area from hematoxylin-stained sections. Quantification of the percentage of area of left ventricle occupied by collagen (%), collagen area was measured in old rats and old rats in CS exposure (Old CSexp). The percentage of tissue attributed to collagen distribution increased more rapidly in old rats in CS exposure than in old rats or young rats (Figure 3A). To detect whether CS exposure led left ventricular hypertrophy exacerbated, we independently calculated the percentage of monocytes per cross-sectional area from hematoxylin-stained sections. Quantification of left ventricular muscle fibers interstitial width of extracellular space, we found left ventricular muscle fibers interstitial become broad resulted in the percentage of extracellular space (%) increase (Figure 3B). During old age or exposure to CS exposure, left ventricle from old rats and old rats in CSexp groups exhibited fewer percentage of per unit area than young rats (Figure 3C). But cardiomyocytes density is increased per unit area than young rats (result not show in this article). Indeed, according to tissue architecture using H&E staining analysis we determined cell size measurement. We observed left ventricular cell size increase width in the old rats. According to left ventricular cell width size, old rats in CS exposure compared with old rats were increased. According to compared with left ventricular cell length size in old rats and old rats in CS exposure were increased (Figure 3E). Compared with old rats, left ventricular cell length size in old rats in CS exposure was significantly increased.

Changes to MMPs protein expression can explain age-related heart failure disease.

Fibrosis occurs from changes in the balance between synthesis and degradation of extracellular matrix components. Therefore, we sought to determine whether aging and CS exposure related collagen accumulation and fibrosis could be related to changes and regulation MMP2 and MMP9 (Figure 4A, 4B and 4D). Gelatin zymography detected 2 major gelatinolytic bands, MMP-2 and MMP-9, in the left ventricular extracts. MMP-9 gelatinolytic bands did not change. As Figure 4A. shown. Because its regulation was the sum of pro-MMP-9 and TIMP-1, it may be MMP-9 complexed with TIMP-1. The down regulation of MMP-2 in old rats and old rats in CS exposure was statistically significant compared with young rats. The extent of changes in MMP-2 gelatinolytic activity was lower than old rats. Consistent with the results of gelatin zymography, MMP-2 and MMP-9 protein content as measured by western blotting was also significantly reduced in old rats and old rats in CS exposure (Old CSexp) (Figure 4B). These results suggest that MMP2/MMP9 (gelatinase) contribute to the remodeling of extracellular matrix in left ventricular fibrosis. While aging, we found MMP2 and MMP9 protein expression decreased. Once exposure to CS exposure, MMP2 and MMP9 protein expression were significantly lower than old rats. Dysregulation of MMP2/MMP9 was now believed to contribute fibrosis in old and aging.

Calcineurin/NFAT pathway is associated with the progression to heart failure in the old CS exposure hypertrophic heart.

Give the above evidence that these changes predispose the aged hearts to the development of left ventricular hypertrophy or heart failure. Molecular mechanisms underlying the progression to heart fibrosis are discussed below. In recent years, the aging has constructed as physiological adaption response which is normal aging process that ultimately leads to pathogenesis. Many aging occurs chronic diseases such as cardiovascular diseases, cancer, diabetes, obesity, atherosclerosis. Calcineurin/NFAT is the Ca^{2+}– dependent serine/threonine protein-phosphatase calcineurin which was identified as a central pro-hypertrophic signaling molecule in the myocardium between in pathological and physiologic. Our data showed continuing activation of calcineurin/NFAT throughout aging and CS exposure induced left ventricular hypertrophy and heart failure (Figure 4C). Calcineurin/NFAT function was to maintain the hypertrophic profile of the left ventricle in old rats and old rats in CS exposure. Interestingly, whereas this result we hope this hypothesis is also supported by the fact that hypertrophy and failing hearts have altered old rats intracellular Ca^{2+} handling, potentially serving as part of the stimulus for calcineurin/NFAT activation.

Elevated TIMPs expression induced fibrosis is present in old rats and old rats in CS exposure.

MMPs catalyze ECM degradation, TIMPs is physiological inhibitors which controlled MMPs activity. To explore whether there is regulation changes in the expression of TIMPs, we assessed the protein expression of TIMPs (TIMP-1, -2, -3 and -4) by Western blot. A up-regulation of TIMPs is associated with heart failure and fibrosis. To determine whether aging or CS exposure modulate cardiac matrix remodeling. As Figure 4D shown, we found TIMP-1, TIMP-2, TIMP-3 and TIMP-4 induced left ventricular fibrosis, protein expression levels by western blotting analysis in old rats and old rats in CS exposure were significantly higher than young rats. Thus, this apparent cause of fibrosis and heart failure can be explained by differential regulation between MMPs and TIMPs.

CS exposure results in higher sensitivity to inflammation, fibrosis, and heart failure in old rats.

CS exposure is an environmental stressor induced pathological LVH. In this study, we determine whether low concentration CS exposure to old rats also will be induced pathological LVH. To further determine the potential of inflammation in old heart, we examined JNK, p38, IL-6, and TNFα protein expression levels by western blotting and immunohistochemistry. As Figure 4E and 4F shown, JNK, p38, IL-6, and TNFα protein expression levels were increased in old rats and old rats in CS exposure. Immunohistochemical study showed that densities of both old rats and old rats in CS exposure hearts were higher in young rats. IL-6 and TNFα play an important role in promoting LVH and inflammation. With greater age comes, we found that MAPK (JNK1/2 and p38) protein expression were increased in old rats and rats in CS exposure. CS exposure may enhance proinflammatory cytokines (IL-6 and TNFα) and MAPK cascade expression in old rats hearts. Thus, cytokines in left ventricular hypertrophy tissues increased markedly in keeping with a denser inflammatory cell infiltration.

Conclusions

Cigarette smoke (CS) has been linked to a number of harmful health outcomes and is an important cause of morbidity and mortality. CS is an important cause of morbidity and mortality. There are a lot of evidences indicated that CS a formidable health hazard. However, there is no evidence indicated that CS exposure presents a challenging health hazard [14]. It is also well understood that toxic air contamination causes lung cancer and cardiovascular diseases. In this study, we investigated the effects of CS exposure associated with the elderly age, specifically in the left ventricles of male rats. As is well known, old age in humans always accompanies an increased incidence of atherosclerotic vascular disease and cardiovascular disease [15]. In contrast, aging is a physiological process due to increasing injuries and vulnerability, which reduces the ability of organisms to survive. Aging affects various aspects of left ventricular morphology and function, and has recently been considered to be a major risk factor for cardiovascular disease and to have effects on various aspects of left vascular morphology and function. Aging can refer to an time-related process, however, it is commonly used for post-maturational processes. The main characteristics associated with aging is a progressive decrease in physiological capacities [16]. The overall affect is highly debatable aging and disease. As age increases, whether there will occurs diseases itself. However, the most obvious evidence of changes in aging heart and liver. Biological phenomena appear related to the aging process [17]. Aging exacerbates cardiac damage, leading to cardiac hypertrophy, fibrosis [18] and dysfunction, developed compensatory concentric hypertrophy [19] and fibrosis in response to induced cardiomyocytes hypertrophy in a similar manner [20]. In aging heart, demonstrated sever left ventricular chamber dilation, wall thinning and fibrosis, leading to congestive heart failure. In this study, we want to know low-level chronic cigarette smoke exposure whether is harmful to left ventricular function in old rats and to explore the related mechanisms. We found that changes associated with CS exposure lead to cardiovascular pathological outcomes resulted in age-related disease exacerbated. We observed left ventricular chamber narrowing and rupture and increased left ventricular wall thickness (Figure 1 and 2). These results demonstrated left ventricular hypertrophy in old rats and old rats in CS exposed (Old CSexp). On the other hand, we could from echocardiography results to determine left ventricular dimension, posterior wall thickness, interventricular septal at end-systole and end-diastole were increased, and left ventricular function declined. Stiffening of these fibers cause left ventricular fibrosis and could also affect the efficient functioning. CS exposure (CS) is linked to a number of harmful health outcomes.

As is well known, CS exposure is a key risk factor for pathological hypertrophy associated with various cardiovascular disease risk factors [21], however, the old annual human always accompany with atherosclerotic vascular disease and cardiovascular disease. As the heart reaches senescence, it undergoes a modest degree of Heart failure [22]. It is now determined the differences several signaling molecules play unique role in regulation of old rats in CS exposure. We discuss molecular signaling mechanisms associated with old rats in CS exposure, including calcineurin/NFAT, MMPs, TIMPs, JNK1/2, p38, IL-6 and TNFα signalings (Figure 4). We suggested that upregulation of pro-inflammatory related protein expression of JNK1/2, p38, IL-6 and TNFα enhanced left ventricular pathological hypertrophy and related proteins, calcineurin and NFAT, expression increases. Down-regulation of MMP2 and MMP9 in old rats in CS exposure accelerated TIMPs-induced cardiac fibrosis. Despite the evidence that chronic exposure to CS results in cardiac changes [23], the exact mechanisms involved in low-level concentration process remain to be elucidated. These explore knowledge may influence therapeutic strategies for the treatment of cardiovascular disease in old age.

Abbreviations

CS Cigarette smoke

LV left ventricles

LVH left ventricular hypertrophy

IVSs Interventricular septal in systemic

IVSd	Interventricular septal in diastole
LVIDd	Left ventricular internal dimension at end diastole
LVIDs	Left ventricular internal dimension at end systemic
LVPWd	Left ventricular posterior wall thickness in diastole
LVPWs	Left ventricular posterior wall thickness in systemic
H&E	Hematoxylin and Eosin staining
MMP 2	matrix metalloproteinase 2
MMP 9	matrix metalloproteinase 9
TIMP-1	tissue inhibitors of metalloproteinases-1
TIMP-2	tissue inhibitors of metalloproteinases-2
TIMP-3	tissue inhibitors of metalloproteinases-3
TIMP-4	tissue inhibitors of metalloproteinases-4

Conflict of interest

The authors have declared that no conflict of interest exists.

Author's contributions

Jia-Ping Wu designed and performed research, and wrote the final paper.

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Captions

Figure 1.

Representative histopathological analysis of left ventricular cross-sections with hematoxylin & eosin (H&E) and masson's trichrome staining in young, old rats and old rats in CS exposure (Old CSexp). (A). Representative by hematoxylin & eosin staining of left ventricular sections in young, old and Old CSexp groups. (B). Representative left ventricular papillary muscles sections by hematoxylin & eosin staining in young, old and old CSexp groups. Scale bars 2mm. The images of left ventricular architectures were magnified 100x. (C). Representative left ventricular extracellular space by hematoxylin & eosin staining in young, old and Old CSexp groups. Scale bars 20μm. The images of left ventricular architectures were magnified 400x. (D). Representative collagen accumulation in the left ventricle by hematoxylin & eosin staining in young, old and Old CSexp groups. Scale bars 20μm. The images of left ventricular architectures were magnified 400x. Yellow arrows express. (E). Representative fibrosis in the left ventricle by masson's trichrome staining in young, old and Old CSexp groups. Scale bars 2mm. The images of left ventricular architectures were magnified 100x. Yellow arrows express. (F). Representative collagen accumulation in the left ventricle by masson's trichrome staining in young, old and Old CSexp groups. Scale bars 20μm. The images of left ventricular architectures were magnified 400x. Yellow arrows express.

Figure 2.

Left ventricular hypertrophy takes place in old rats and old rats in CS exposure (Old CSexp).

(A). Quantification of heart weight and left ventricle weight statistical analysis. $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats. (B). Quantification of the heart weight to body ratio and the ratio of left ventricular weight to body weight statistical analysis. $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats. (C). Quantification of heart weight to tibial ratio and the ratio of left ventricular weight to tibial statistical analysis. $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats. (D). Representative M-mode echocardiograms taken proximal from young, old and Old CSexp. These images were obtained from short- axis imaging view at the midpapillary level. Parasternal long-axis echocardiography views (up-panel), parasternal short-axis echocardiography views (down-panel). Interventricular septal in systolic (IVSs), interventricular septal in diastolic (IVSd), left ventricular internal dimension at end systolic (LVIDs), left ventricular internal dimension at end diastolic (LVIDd), left ventricular posterior wall thickness in systolic (LVPWs) and left ventricular posterior wall thickness in diastolic (LVPWd) shown in right panel. (E). Quantification of interventricular septal at diastolic and systolic, left ventricular internal dimension at end diastolic and systolic, left ventricular posterior wall thickness at diastolic and systolic, and the percentage of fractional shorting and ejection fraction. $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats.

Figure 3.

Histopathologic of left ventricular fibrosis and left ventricular hypertrophy in young, old rats and old rats in CS exposure (Old CSexp).

(A). Quantification of percent of area of left ventricle occupied by collage. $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats. (B). Quantification of percent (%) extramyocyte connective tissue space (area). $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats. (C). Quantification of average number of myocytes per 400μm. Values were calculated from myocardial regions. $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats. (D). Using histological sections by H&E stained to determine cell width (μm) and cell length (μm) in young, old rats and old rats in CS exposure (Old CSexp). (C). Quantification of cell width (μm) and cell length (μm) in young, old rats and old rats in CS exposure (Old CSexp). $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats.

Figure 4.

Molecular mechanisms of the unbalance of MMPs and TIMPs induced fibrosis and ECM remodeling.

(A). Representative zymographical analysis from young, old rats and old rats in CS exposure (Old CSexp). Up-panel shows a gelatin zymography graphic representative of MMP-2 and MMP-9 activity in young, old and old rats in CS exposure. Down-panel shows MMP-2 activity quantified by densitometry and expressed as mean pixel density. Values are represented as the means ± SEM. $^{*}p<0.05$ compared with young rats. $^{\#}p<0.05$ compared with old rats. (B). MMP-2 activity dysregulation and are in old rats and old rats in CS exposure (Old CSexp) by wester blotting. (C). Calcineurin and NFAT protein expression levels are increased by western blotting analysis. (D). TIMP-1, TIMP-2, TIMP-3 and TIMP-4 protein expression elevated in old rats and old rats in CS exposure (Old CSexp). (E) Immunohistochemistry of IL-6, TNFα, JNK and p38 in young, old rats and old rats in CS exposure.

Figure 5.

A schematic illustration of the effects of cigarette smoke (CS) exposure on the activation of molecular mechanisms of inflammation and fibrosis during aging. Upregulation of the expression of inflammatory mediators, cytokines (IL-6 and TNFα) and MAPKs (JNK1/2 and p38), which leads to inflammation response. Upregulation of TIMPs lead to collagen accumulation and fibrosis, which resulted in ECM degradation reduced and down regulation of MMP-2 activity in old rats in CS exposure.

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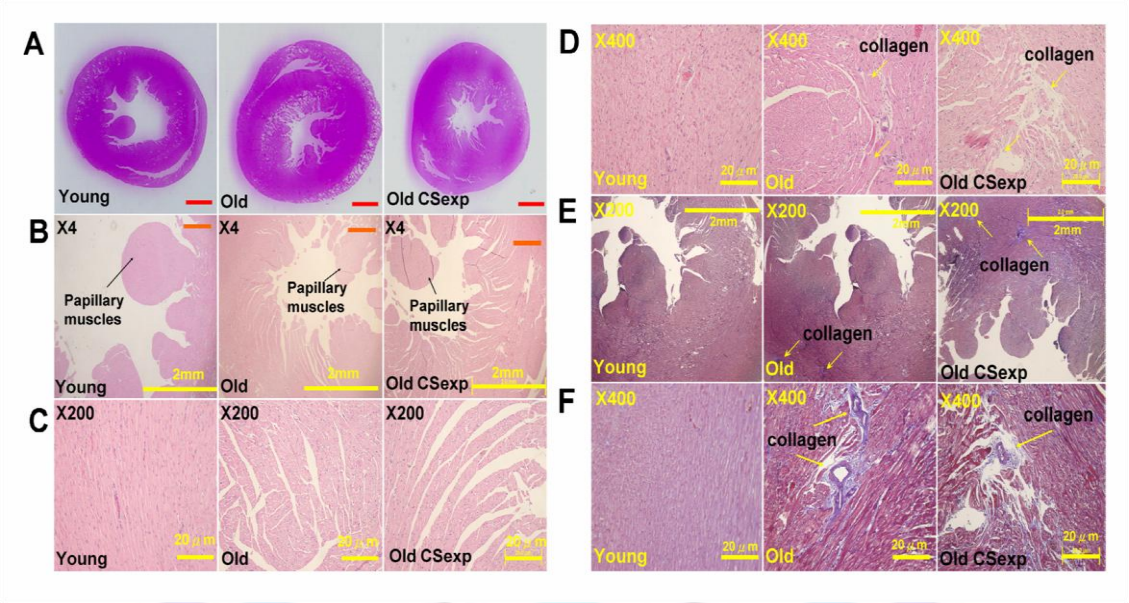
Figure 1.



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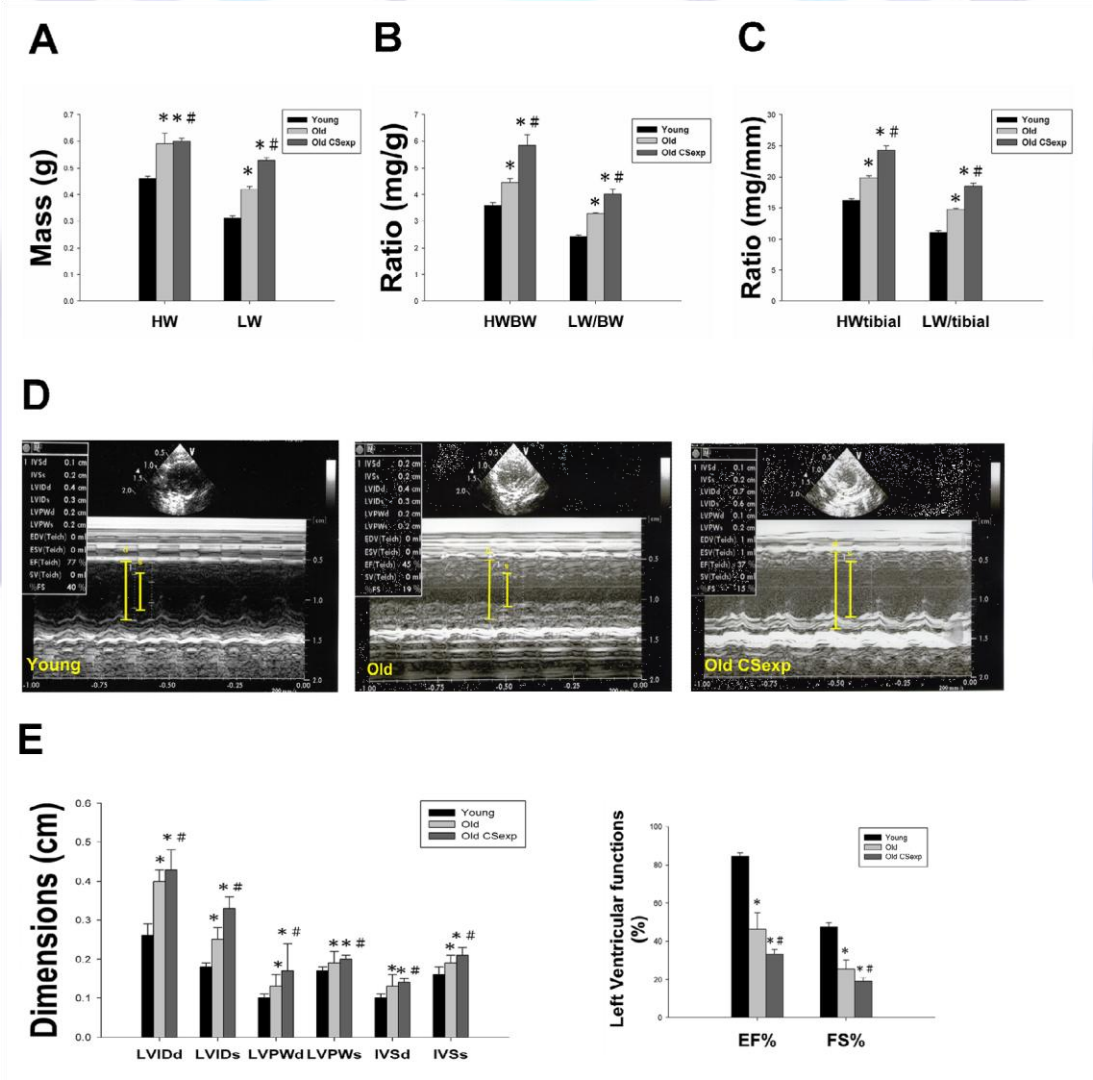

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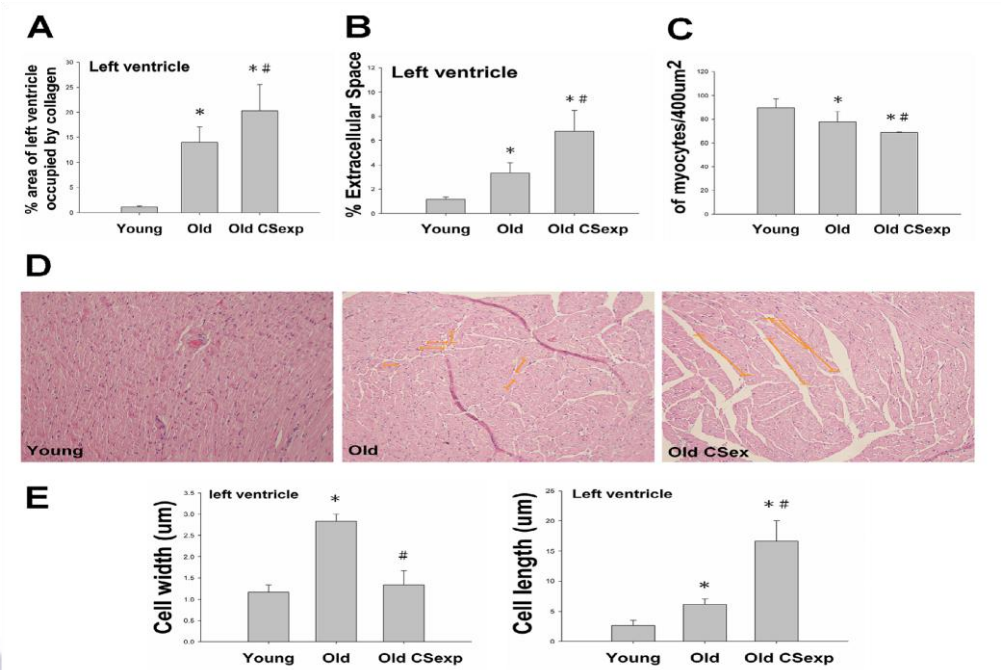
Figure 3.



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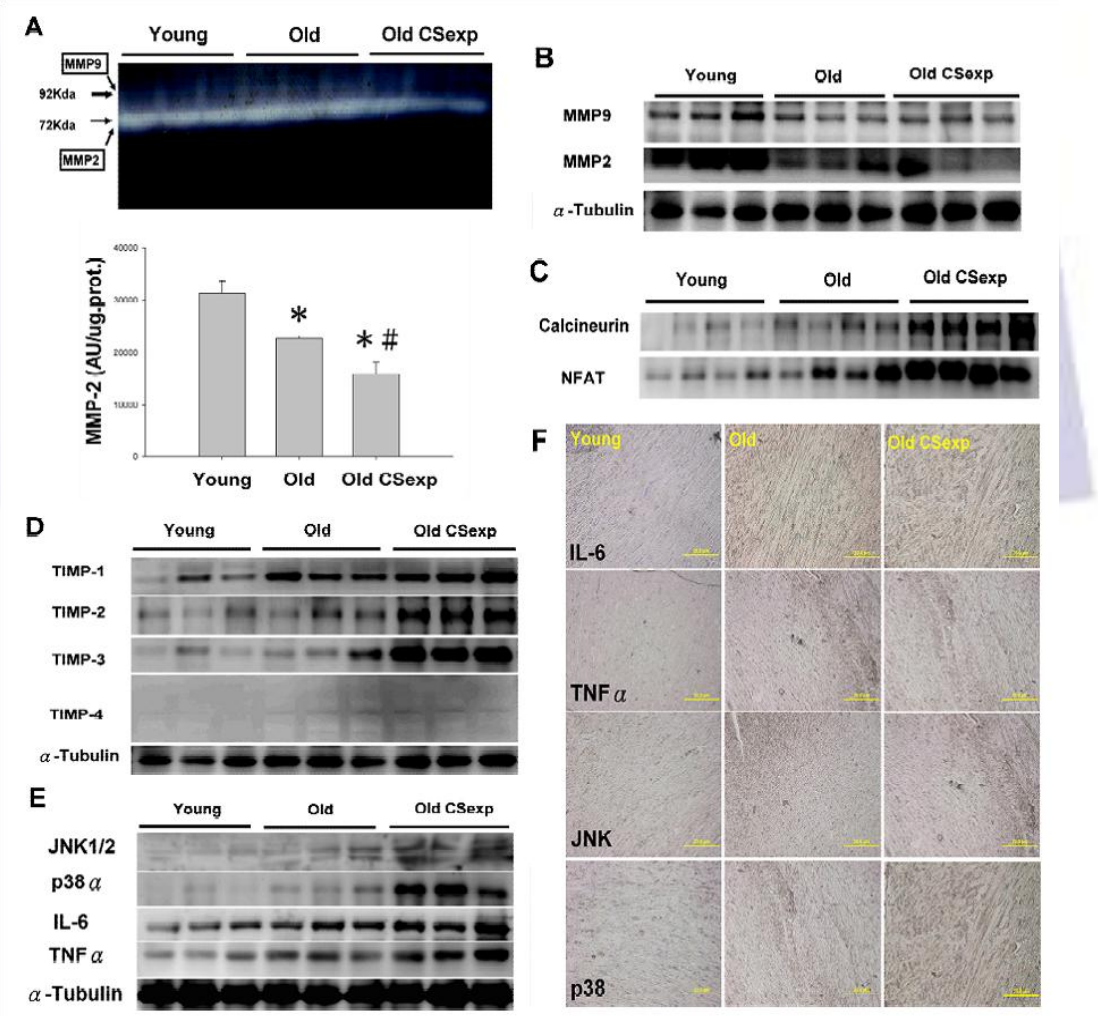

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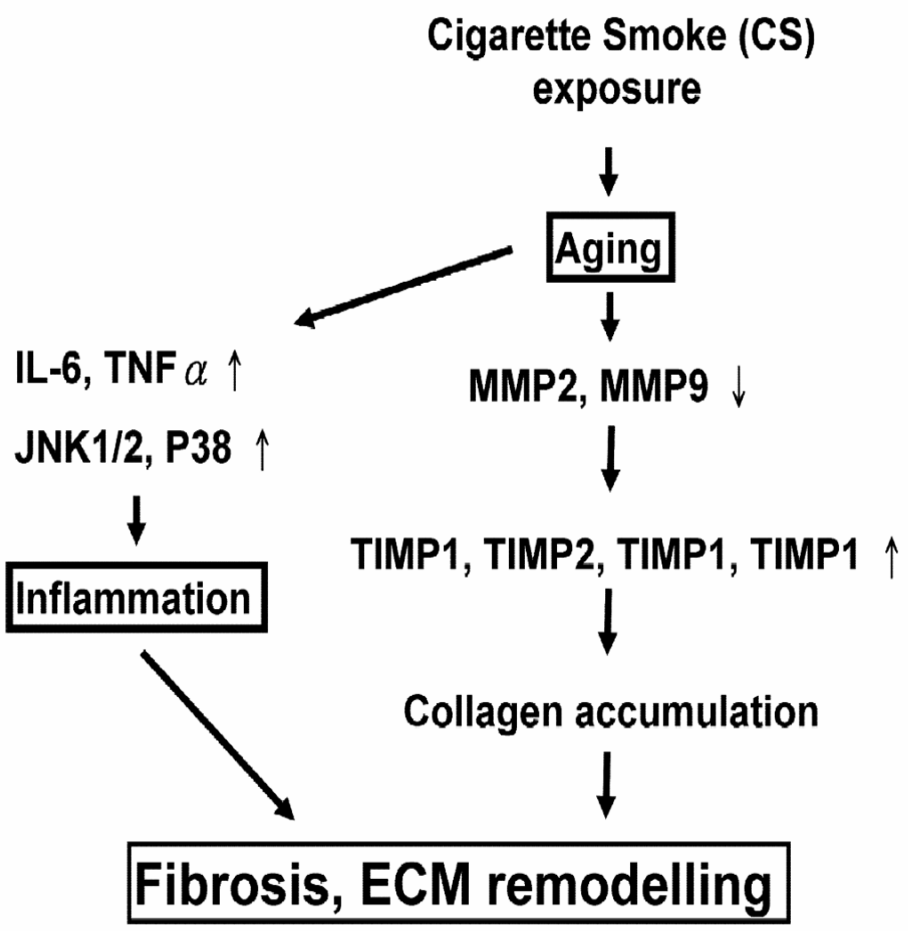
Figure 5.