Original Article

Senescent Ventricular Dysfunction: Issues Related to Cardiopulmonary Bypass

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ABSTRACT

The mean age of the open-heart surgical patient is increasing every year. Therefore, it is logical to define the aging-related changes in cardiovascular function. This study set forth to define the major molecular and performance alterations that occur in the left ventricle related to advanced aging or senescence. In the human, vascular pathologies usually accompany left ventricular dysfunction. The aim of this study was to associate the altered left ventricular mechanics with molecular pathways in mice who lacked these associated vascular pathologies. This study compared the left ventricular function of two groups of mice ($N = 20$ each), 6 months old and 16 months old (senescent). The mice were anesthetized with urethane and α-chloralose, and a Millar 1.4 Fr. conductance micromanometer catheter was placed into the left ventricle for acquisition of pressure-volume loops. Heart tissues were collected immediately for analysis of cGMP concentrations. The cardiac index, preload recruitable stroke work, and the slope (Ees) of the end-systolic pressure volume relationship were significantly less in the senescent group compared to the young mice. It was concluded that the aged heart has significantly reduced systolic and diastolic dysfunction compared to the young heart function and that this dysfunction may be related to pathways leading to increased myocardial cGMP concentrations.

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INTRODUCTION

Epidemiological studies have shown that heart disease is an increasingly major contributor of mortality in the over 65 population. Therefore, the population requiring open-heart surgical procedures is also increasing proportionally. The fundamental biological process of cardiac senescence in the human has been associated with decreased vascular compliance, vascular disease, and changes in physical activities (1). However, there appears to be an intrinsic change in the ventricular mechanics and molecular function that is independent of the senescence of the vasculature (2). From a biological viewpoint, cardiac senescence results from four different factors: intrinsic cardiac processes, endocrine system senescence, immune senescence, and vascular senescence. The goal of this research was to compare the ventricular mechanics of young mice against older mice lacking the vascular senescence.

The senescent heart, in the absence of arterial hypertension and coronary insufficiency, provides the opportunity to define the contributory roles of the immune system and the endocrine system to senescent heart dysfunction. The endocrine system is altered with aging as exhibited by an increased release of atrial natriuretic factors (ANF) and the renin angiotensin system (RAS) (3). The failing heart is known to over-express tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-β) (4). These same cytokines most likely play a pivotal role in the senescent dysfunction. At a molecular level, there is a phenotypic change in contractile protein expression and calcium cycling proteins (2). The calcium cycling proteins include L-type calcium channel, ryanodine receptor, SERCA2a, phospholamban, and calsequestrin, as illustrated in Figure 1. Furthermore, we have shown that the contraction efficiency of the senescent heart in much less than that of the younger heart (5). The overall effect of these changes in senescent individuals is a decreased basal level of cardiac function coupled with a reduced ability to respond to stress situations, as is encountered with open-heart surgical procedures.

The analytical system that has been developed recently to quantify the left ventricular function in mice is the conductance catheter system (CCS) (5). This system can provide pressure-volume loops (PVL) of the left ventricle with a high degree of fidelity and permit the quantitation of systolic, diastolic, ventricular elastance, and ventricular efficiency under pre-load independent conditions. We employed the CCS in the in situ mouse model and compared the functional parameters of young and moderately old mice. Furthermore, the molecular factor cGMP may be the pivotal factor in inhibition of the calcium cycling proteins in the myocyte, as shown in Figure 1. Therefore, we determined the relationship between cGMP and heart function. In summary, our hypothesis was that there is an age-dependent decrease in pre-load independent left ventricular parameters that parallels the myocyte concentrations of cGMP.

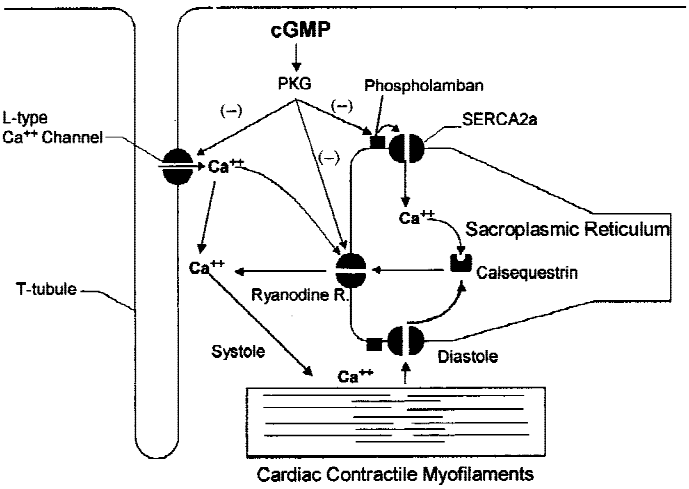

Figure 1: The pathways regulating calcium in the cardiac myocyte. Systolic function is directly related to the quantity of calcium released from the L-type calcium channels and the sarcoplasmic reticular stores that are controlled by the ryanodine receptor. Diastolic function is related to the reuptake function of the SERCA2a that is regulated by the protein phospholamban. Elevated cGMP can reduce the calcium influx subsequent to an action potential and affect the amount of calcium released from the sarcoplasmic reticulum. The elevated cGMP may affect the affinity of calcium on the contractile fibers and also reduce the function of the proteins that regulation calcium reuptake into the sarcoplasmic reticulum, namely SERCA2a and phospholamban.

MATERIALS AND METHODS

ANIMALS

Forty C57BL/6 male mice were purchased from National Institute for Aging, (Washington, DC) and divided into two groups. Group I ($N = 20$) consisted of 6-month-old mice and Group II ($N = 20$) consisted of 16-month-old mice (senescent). The mice were anesthetized with urethane in saline (1000 mg/kg, i.p.) and α-chloralose in propylene glycol (50 mg/kg, i.p.). The mice were ventilated through a tracheostomy connected to a pressure-controlled respirator[a] at a rate of 120 times/min and FiO_2 of 1.0. The apical portion of the heart and the inferior vena cava (IVC) were exposed through a substernal-transverse incision. A Millar Conductance Catheter 1.4 Fr[b] was inserted into the apex of the left ventricle and with the distal electrode in the aortic root and the proximal electrode in the LV apex. The pressure-volume relationships were performed with a Millar 1.4 F catheter (SPR-719) that was a composite of four conductance electrodes and a micromanometer. The distance between the conductance sensor electrodes of this catheter was 4.5 mm approximating the distance between the cardiac apex and the aortic valve. The sampling rate

[a] RSP 1002, Kent, CT
[b] Millar Corporation, Houston, TX

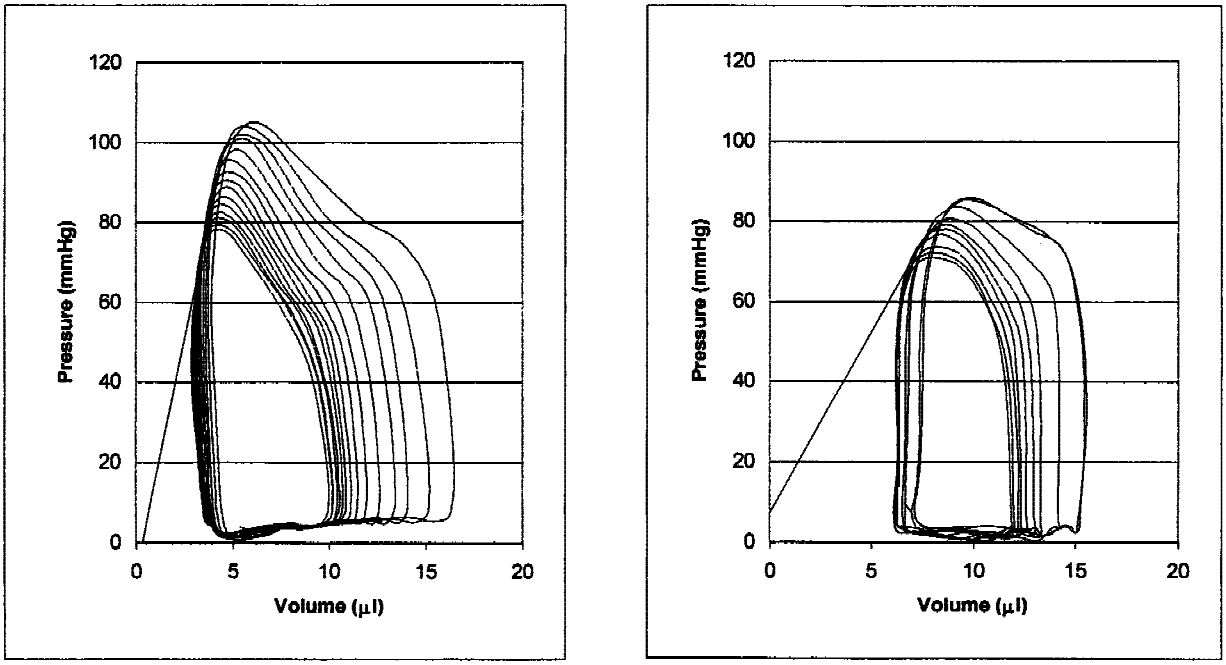

Figure 2: The PVL demonstrate a significantly reduced cardiac performance in the aged mice. The ramping of the PVL was performed to acquire pre-load and afterload independent parameters of contractility (PRSW), ventricular elastance (β), and contractile efficiency (the area under the plotted line). The figure on the left represents the younger group and the figure on the right represents the older group.

was set at 1000 Hz to acquire 110 points per loop. The conductance and pressure signals were digitized with the software, BioBench[c], stored in Excel, and analyzed with Pvan version 6.2[d]. To acquire pre-load independent measurements, the inferior vena cava was gently compressed for 2 sec while continuously recording the PVL. The pre-load independent parameters of contractility and diastolic function were measured from these ramped pressure-volume loops, including Ees from ESPVR, PRSW, and β from EDPVR. The peripheral vascular resistance (Ea), cardiac output, ejection fraction, dP/dt_{max} and dP/dt_{min} and were acquired during the non-occluded state.

cGMP ENZYME IMMUNOASSAY

The Biotrak cGMP Competitive Enzyme Immunoassay System[e] was utilized to quantify the left ventricular concentration of cGMP. This assay is centered on competition between unlabelled cGMP and a fixed quantity of peroxidase-labelled cGMP for a limited number of binding sites on a cGMP antibody. Incubation of the antibody-antigen mixture was for 3 h and the reaction was stopped with dodecyltrimethylammonium bromide and incubated for 30 min. A blue color developed and was read in triplicate in a microliter plate reader at 630 nm. The intracellular cGMP concentrations per well were computed from a standard curve. The cGMP concentrations were expressed as fmol/gram of wet heart weight.

STATISTICAL ANALYSIS

All data are reported as mean ± SEM. The standard volume line was analyzed by simple linear regression. When appropriate, differences between young mice and senescent mice were compared by an unpaired Student's *t*-test, or one-way ANOVA. The effects of AMG and MITU were compared with a paired Student *t*-test. $p < .05$ was used as criteria for statistical significance.

RESULTS

The PVL in Figure 2 shows the functional difference between the young and older mice. The PVL were used to describe the parameter Ees, which is the slope of the end systolic pressure volume relationship (ESPVR). The Ees was significantly different between the young and old mice with 26.3 ± 2.8 and 15.5 ± 1.7 mmHg/μL ($p < .001$), respectively. The ESPVR + PVL area plot, shown in Figure 2, describes that the contractile efficiency, which was much less in the older group compared to the young group. The pre-load dependent parameters of ejection faction, cardiac index, dP/dt_{max}, and dP/dt_{min} shown in Table 1 and Figure 3 are all significantly reduced in the older group. Figure 4 shows that the pre-load recruitable stroke work (PRSW) was significantly less in the older group

[c] National Instruments, Austin, TX
[d] Conductance Technologies Inc., San Antonio, TX
[e] Amersham Pharmacia Biotech, Buckinghamshire, UK

($p < .0001$). This parameter is a robust measurement of systolic function due to the incorporation of the complete PVL in the computation of its slope. Secondly, it does not require a precise volume calibration as do the other described parameters (5). The ventricular elastance, β, was significantly higher in the old group compared to the young by 46%. These data demonstrated that the left ventricle was less compliant at any given ventricular volume. In contrast, the peripheral vascular resistance, Ea, did not differ between the two groups. These data demonstrated a decreased systolic, diastolic function, and ventricular elastance without differences between the peripheral vascular resistance.

Subsequent to the hemodynamic measurement, myocardial tissues were harvested and processed for cGMP concentrations. This study shows that there is a significant increase in cGMP concentrations ($p < .01$) in the old group compared to the young group (Figure 5). These data support our hypothesis that the increased cGMP may account for the decreased ventricular function possibly through the inhibition of the calcium cycling proteins as illustrated in Figure 1.

DISCUSSION

These data provide evidence that there is a decrease in ventricular function related to age and that the pivotal pathway through cGMP may possibly be the mechanism. The present results do not define the mechanism for the elevated cGMP concentrations. However, they do define that without increased peripheral vascular hypertension there is senescent cardiac dysfunction. These data do provide the opportunity to examine the immune and endocrine pathways as mediators for the elevation in cGMP. Moreover, these data also underscore the need of the perfusionist, anesthesiologist, and surgeon to understand that senescent patients are at risk for poor cardiac function and possibly poor outcomes.

There is much supportive data to suggest that the calcium cycling protein function is decreased secondary to aging. The aggregate age-associated alterations in cytosolic calcium concentrations causes the cardiac dysfunction. A decrease in the L-type calcium channel and ryanodine regulatory protein of the sarcoplasmic reticulum may account for the decreased systolic

Table 1:

Parameter	Young	Old	*p*
Body weight (g)	31.7 ± 0.6	35.5 ± 0.6	ns
Heart rate (bpm)	582 ± 8	537 ± 11	ns
Ejection fraction (%)	65 ± 2	54 ± 2	0.001
Cardiac index (μL/g)	221 ± 10	168 ± 15	0.001
Ventricular elastance (β) (mmHg/μL)	0.26 ± 0.04	0.38 ± 0.07	0.05
Arterial elastance (Ea) (mmHg/μL)	8.4 ± 0.7	7.3 ± 0.5	ns

The ejection fraction and cardiac index of the older mice was significantly reduced compared to that of the young. The ventricular elastance was significantly higher in the older mice implying that the ventricular compliance was less. However, the peripheral vascular resistance as described by Ea did not differ between the two groups.

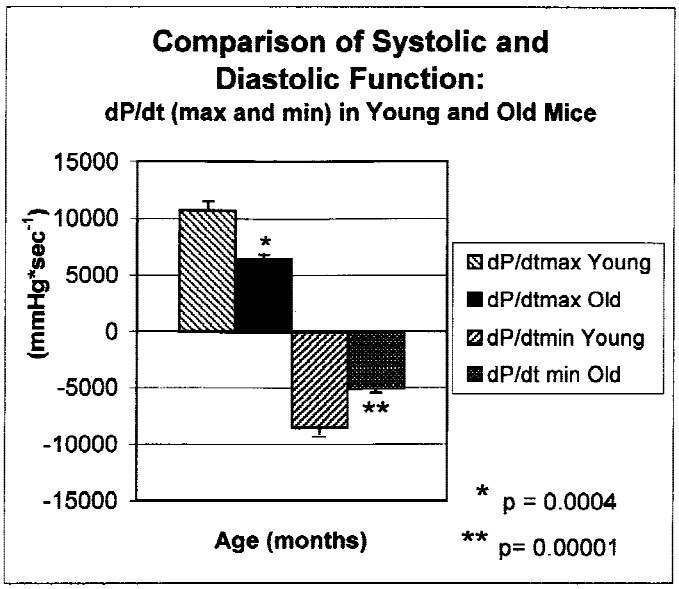

Figure 3: The dP/dt_{max} was significantly less in the older group, implying rate of systolic contractility was less compared to the younger. The dP/dt_{min} was also less in the old group, suggesting that the diastolic function of the older group was less than the younger. These parameters are influenced by the pre-load and afterload of the heart.

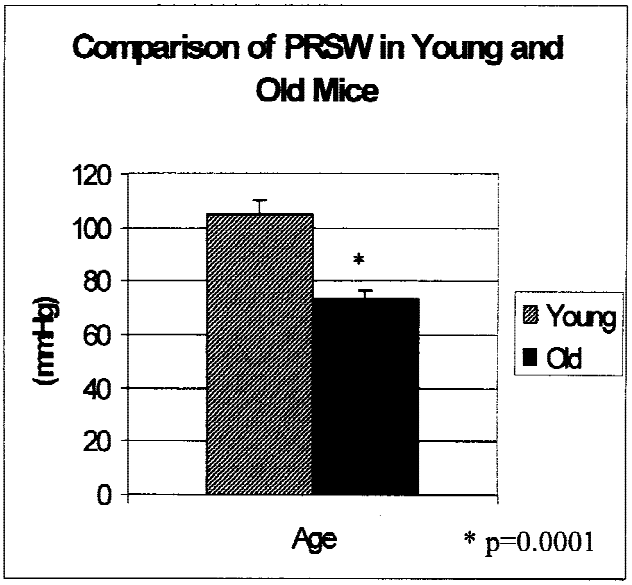

Figure 4: The PRSW was significantly decreased in the older group. This parameter is independent of pre-load and afterload and therefore describes the ventricular mechanics independent of peripheral vascular volumes and pressures.

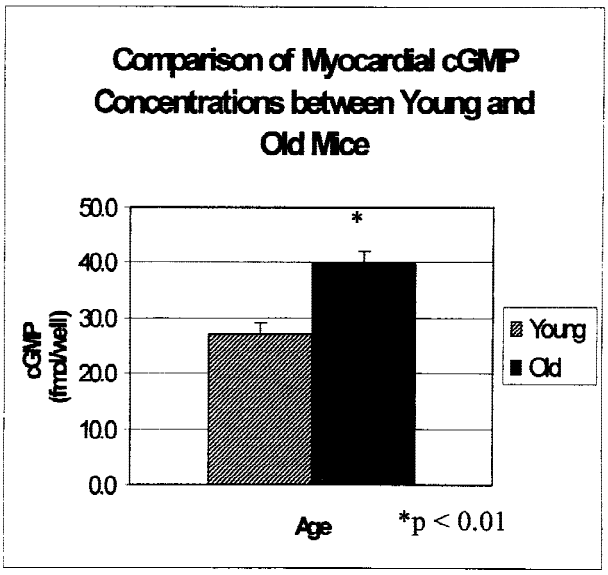

Figure 5: The myocardial cGMP content was significantly increased in the older group. These data suggest that there is a molecular basis for the reduced contractile function in the aged possibly related to the negative regulation of calcium cycling proteins in the cardiac myocyte.

function in the senescent individual (2). Also, the reuptake protein SERCA2a function is decreased in the aged human and rodent heart that accounts for decreased diastolic function (6). Furthermore, the β-receptor-mediated stimulation of the myocyte is reduced in the aged due to this decrease in calcium transient (7). The common pathway that may down regulate these protein functions may be cGMP.

The strength of this study linking ventricular function with cGMP was that the findings were acquired in the whole animal. Others have demonstrated the relationship between cGMP and contractility in preparations of isolated hearts in a Langendorff preparation or isolated papillary muscle preparations. Most reports suggest that increased levels of cGMP are associated with depressed contractility (8–11). More specifically, increased cGMP inhibits the ryanodine regulatory protein, phospholamban, and appears to oppose the enhancing effects of cAMP (12) The enzyme cGMP dependent protein kinase mediates the cGMP effects on these calcium cycling proteins. The logical question related to our findings is to determine what endocrine and immune factors stimulate the cGMP.

In other states of cardiac dysfunction, increased myocyte derived TNF-α is associated with cardiac dysfunction. In viral myocarditis, cardiac allograft rejection, cardiac ischemia, and sepsis there is a significantly increased level of TNF-α (13, 14). The myocardium appears to be a major source of TNF-α subsequent to cardiopulmonary bypass (15, 16). Antagonist to TNF-α prevent the post-cardiopulmonary bypass myocardial depressions (17). One main target for TNF-α is the induction of inducible nitric oxide synthase (iNOS). Most importantly, iNOS produces NO directly proportional to the amount of enzyme contained per cell (18). The effect of elevated NO is the generation of cGMP by soluble guanyl cyclase (sGS). The resulting increased NO mediated cGMP does result in depressed myocardial function (19). Therefore, it is possible that the immune system releases increased TNF-α due to senescence resulting in elevated cGMP through the nitric oxide pathway.

CONCLUSION

We have shown that there is a significant depression in systolic and diastolic function in an in situ model of senescence. This cardiac dysfunction appears to be related to the elevation of cGMP in the myocardium. Based on these data, the senescent open-heart surgical patient would have a diminished cardiac reserve and possibly a reduced response to inotropic pharmacological support. A number issues appear to evolve from this study namely; the need to conduct perfusion-related to age, the need to reassess myocardial preservation in the aged, and the need to further define the molecular basis for the observed senescent cardiac dysfunction.

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