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Aminoguanidine prevents age-related aortic stiffening in Fisher 344 rats: aortic impedance analysis

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1 We determined the effects of long-term treatment with aminoguanidine (AG), an inhibitor of advanced glycation end products, on the mechanical properties of the arterial system in aged Fisher 344 rats, using the aortic impedance analysis.

2 Normotensive rats were treated from 18 to 24 months with AG ($1\,g/l^{-1}$ in drinking water) and compared with a control group. Pulsatile aortic pressure and flow signals were measured and then subjected to Fourier transformation for the analysis of aortic input impedance. Wave transit time was determined using the impulse response function of the filtered aortic input impedance spectra.

3 With no alteration in body weight, rats treated with AG had decreased heart weight compared with the aged untreated controls.

4 AG did not affect arterial blood pressure; however, the age-related increase in total peripheral resistance was prevented by AG.

5 AG retarded the age-related decline in aortic distensibility, as evidenced by a reduction of 25.2% in aortic characteristic impedance and an increase of 28.1% in wave transit time.

6 Meanwhile, the increase in wave reflection factor in aging rats was reduced by 32.3% by AG. Both the increased wave transit time and the decreased wave reflection factor suggest that AG may prevent the age-related augmentation in systolic loading condition for the left ventricle coupled to the arterial system.

7 We conclude that long-term treatment with AG may impart significant protection against aortic stiffening and cardiac hypertrophy in aged Fisher 344 rats.
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Keywords: Advanced glycation end products; AG; aortic input impedance; aortic distensibility; pulsatile wave reflection

Abbreviations: AG, Aminoguanidine; AGEs, advanced glycation end products; BW, body weight (g); C, systemic arterial compliance ($\mu l\,kg^{-1}\,mmHg^{-1}$); CO, cardiac output ($ml\,kg^{-1}\,min^{-1}$); HR, basal heart rate (beats min^{-1}); iNOS, inducible isoform of nitric oxide syntheses; LVW, left ventricular weight (mg); NO, nitric oxide; P_b, magnitude of the backward pressure (mmHg); P_d, diastolic aortic pressure (mmHg); P_f, magnitude of the forward pressure (mmHg); P_m, mean aortic pressure (mmHg); P_s, systolic aortic pressure (mmHg); R_f, wave reflection factor; R_p, total peripheral resistance (mmHg min $kg\,ml^{-1}$); SV, stroke volume ($ml\,kg^{-1}$ $beat^{-1}$); Z_c, aortic characteristic impedance (mmHg min kg ml^{-1}); Z_i, aortic input impedance spectra; τ, wave transit time (ms)

Introduction

Aging is associated with cardiac hypertrophy and arterial stiffening possibly associated with glycation of proteins and production of advanced glycation end products (AGEs) (Schnider & Kohn, 1980; Vlassara *et al*., 1992; 1994). AGEs are a complex and heterogeneous group of compounds that have been shown to accumulate slowly in vascular and renal tissues with age, and at a more rapid rate in diabetes (Brownlee *et al*., 1988; Vlassara *et al*., 1994). Despite their complexity and widespread pathological distribution, AGEs help in the formation of covalent crosslinks between proteins, which are thought to be one of the central underlying processes by which they cause damage (Bucala, 1997). The pathological cross-linking of subendothelial structural proteins such as collagen can affect tissue remodeling and result in activation of smooth muscle proliferation with loss of elasticity (Brownlee *et al*., 1988). Thus, the age-related increase in collagen crosslinking and accumulation of AGEs may contribute to the development of certain physical changes of the aging vasculature.

Therapeutic interventions for reducing AGEs formation should target AGEs formation by reducing crosslink formation (Bierhaus *et al*., 1998; Kass *et al*., 2001; Vaitkevicius *et al*., 2001). Much evidence has shown that the AGEs inhibitor AG may reduce tissue AGEs accumulation and impart significant protection against the adverse physiochemical and biological effects in aging (Li *et al*., 1996; Corman *et al*., 1998) or experimental diabetes (Brownlee *et al*., 1986; Soulis-Liparota *et al*., 1991). A recent study, which was conducted by Cantini *et al*. (2001), demonstrated that rats treated with AG from 20 to 30 months had reduced aortic wall stiffness, with no alteration in wall stress or scleroprotein composition. Their data suggest that AG decreases the formation of aortic collagen crosslinks produced by the formation of AGEs.

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Whereas no vascular impedance was presented in that study on AG to describe the arterial load imposed on the aged heart. We now determine the role of AG in the modulation of age-related changes in the pulsatile nature of blood flows in arteries, using the aortic impedance analysis (Milnor, 1989; Nichols & O'Rourke, 1998; Chang *et al*., 2003).

The aortic input impedance spectrum (Z_i) is the frequency relationship between the pressure and the flow signals measured in the ascending aorta. In a hydraulic vascular system, the ratio of pulsatile aortic pressure to flow is termed the aortic characteristic impedance (Z_c) if only centrifugal waves are present at origin (Milnor, 1989; Nichols & O'Rourke, 1998). Z_c is directly related to the blood density and pulse wave velocity and is inversely related to the lumen radius squared of the tube. For large arteries, pulse wave velocity has an inverse relation to the distensibility of the aortic wall (Milnor, 1989; Nichols & O'Rourke, 1998). As a consequence, Z_c has been frequently used as an indicator of aortic stiffness: the higher the aortic characteristic impedance, the stiffer the aortic wall. Meanwhile, wave transit time (τ), which is the time for a wave to propagate from one end of the vasculature to the other, could be derived to describe the timing of pulse wave reflection from the peripheral circulation (Milnor, 1989; Chang *et al*., 2003).

In this study, normotensive Fisher 344 rats were treated from 18 to 24 months with AG (1 g l^{-1} in drinking water) and compared with a control group. The results indicated that the aging process causes a decline in aortic distensibility and an early return of pulse wave reflection from the peripheral circulation, leading to an augmentation in systolic loading condition for the left ventricle (LV) coupled to the arterial system. It is very likely that the prevention of age-related cardiac hypertrophy by AG treatment corresponds to the drug-induced decline in LV afterload.

Methods

Animals and catheterization

The specific pathogen-free male Fisher 344 rats at the ages of 6 ($n=9$), 18 ($n=9$), and 24 ($n=18$) months were used to determine the effects of aging and AG on the mechanical properties of the arterial system. Animals were obtained from the colony maintained in the barrier facilities at the Animal Center of Medical College, National Taiwan University. All animals were allowed free access to the Purina chow and water and housed two to three per cage in a 12-h light/dark cycle animal room. Periodic checks of the cages and body weights ensured that the food was administered properly. At the age of 24 months, rats were randomized into two groups, control ($n=9$) and experimental ($n=9$). Animals in the experimental group were treated from 18 to 24 months with AG and compared with the control group. AG hemisulfate (Sigma) was added to the drinking water (1 g l^{-1}) in the experimental group. The water consumption of the animals was checked each week. Control rats drank 16.7 ± 0.4 ml day^{-1} (mean $\pm$ s.e.), and treated rats drank 18.9 ± 0.5 ml day^{-1}. These values were not significantly different and were constant throughout treatment. The animal experiments were conducted according to the *Guide for the Care and Use of Laboratory Animals*, and were approved by the Animal Care and Use Committee of the National Taiwan University.

General surgical procedures and measurement of the hemodynamic variables in anesthetized rats have been described (Chang *et al*., 2003). In brief, rats were anesthetized with sodium pentobarbital (35 mg kg^{-1}, i.p.), placed on a heating pad, intubated, and ventilated with a rodent respirator (Model 131, New England Medical Intruments, Medway, MA, U.S.A.). The femoral vein was cannulated for the administration of supplemental pentobarbital (30 mg kg^{-1} every 2 h). The chest was opened through the second intercostal space of the right side. An electromagnetic flow probe (model 100 series, internal circumference 8 mm, Carolina Medical Electronics, King, NC, U.S.A.) was positioned around the ascending aorta to measure the pulsatile aortic flow. A high-fidelity pressure catheter (model SPC 320, size 2F, Millar Instruments, Houston, TX, U.S.A.) was used to measure the pulsatile aortic pressure *via* the isolated carotid artery of the right side. The electrocardiogram (ECG) of lead II was recorded with a Gould ECG/Biotach amplifier (Cleveland, OH, U.S.A.). The selective pressure and flow signals of 5–10 beats were averaged in the time domain, using the peak R wave of ECG as a fiducial point (Figure 1a, b). Timing between the pressure and flow signals, due to spatial distance between the flow probe and proximal aortic pressure transducer, was corrected by a time-domain approach, in which the foot of the pressure waveform was realigned with that of the flow (Mitchell *et al*., 1994). The resulting pressure and flow signals were subjected to further vascular impedance analysis.

Aortic input impedance spectra

The aortic input impedance (Z_i) could be obtained from the ratio of ascending aortic pressure harmonics to the corresponding flow harmonics (Figure 1c, d), using a standard Fourier series expansion technique (Milnor, 1989; Nichols & O'Rourke, 1998; Chang *et al*., 2003). Total peripheral resistance of the systemic circulation (R_p) was calculated as mean aortic pressure divided by mean aortic flow. The aortic characteristic impedance (Z_c) was computed by averaging high-frequency moduli of the aortic input impedance data points (Huijberts *et al*., 1993; Gaballa *et al*., 1999). Taking Z_c into consideration, we calculated the systemic arterial compliance C ant any pressure P by expanding the two-element (Liu *et al*., 1986) into the three-element Windkessel model, which accounts for a nonlinear exponential pressure–volume relationship:

$$C(P) = \frac{SV\times b}{K + Z_c\times SV/A_d}\times\frac{e^{b\times P}}{e^{b\times P_i} - e^{b\times P_d}}$$

where SV is the stroke volume; K is the ratio of total area under the aortic pressure curve to the diastolic area (A_d); b is the coefficient in the pressure-volume relation (-0.0131 ± 0.009 in aortic arch); P_i is the pressure at the time of incisura and P_d is the end-diastolic pressure (Liu *et al*., 1986; Chang *et al*., 2003).

The wave transit time (τ) can be computed by the impulse response of the filtered Z_i (Figure 2). This was accomplished by the inverse transformation of Z_i after multiplication of the first 12 harmonics by a Dolph–Chebychev weighting function with the order 24 (Laxminarayan *et al*., 1978). When the time of the initial peak (the short arrow in Figure 2) is identified as the reference, the second peak (the long arrow in Figure 2) can be regarded as the standard for the calculation of wave transit

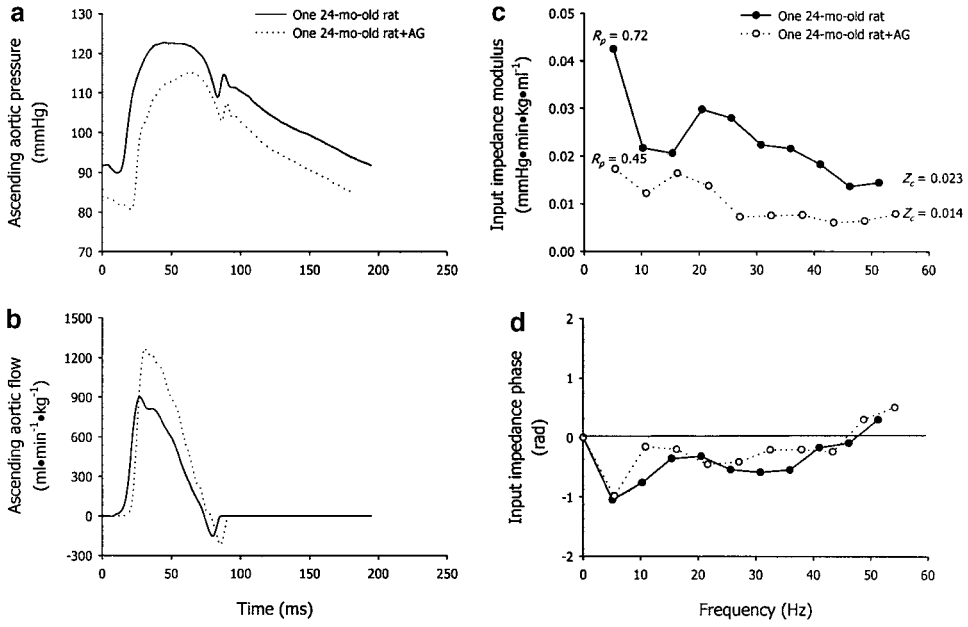

Figure 1 Left: Ensemble averaged pressure and flow waveforms from a rat treated with AG (dashed lines) compared with those of an untreated old animal (solid lines). Right: Aortic input impedance spectra derived from the ascending aortic pressure and flow signals shown in the left panel. The input impedance modulus of aging was displaced upward because of the impaired wall elastic properties and to the right owing to diminished aortic distensibility. AG prevented age-related aortic stiffness, without affecting arterial blood pressure.

time in the lower body circulation (Sipkema *et al*., 1980; Latson *et al*., 1987). Meanwhile, the time domain reflection factor (R_f) can be derived as the amplitude ratio of backward-to-forward peak pressure wave by the method Westerhof *et al*. (1972) proposed. Therefore, both the wave transit time and the wave reflection factor may characterize the wave reflection phenomenon in the vasculature.

Statistics

Results are expressed as means$\pm$s.e. Since cardiac output is significantly related to body shape, this variable was normalized to body weight to calculate the aortic input impedance. Analysis of variance (ANOVA) was used to determine the statistical significance, while multiple comparisons were made for the effects of aging and AG on the arterial mechanics. Significant differences were assumed at the level of $P<0.05$. If ANOVA for a hemodynamic variable reached the significant level, then Tukey's honestly significant difference (HSD) method was used to determine the groups of rats having different mean values of the variable.

Results

Table 1 shows the effects of aging and AG on body weight, left ventricular weight (LVW) and aortic pressure profile. Rats treated with AG did not differ in body weight from untreated controls; however, the significant age-related increase in LVW was not observed in the corresponding AG-treated group. Treatment with AG from 18 to 24 months produced no alteration in the blood glucose level between the 24-month-old controls ($103.8\pm6.4\,mg\,dl^{-1}$) and the 24-month-old treated rats ($112.4\pm5.9\,mg\,dl^{-1}$). Aortic pressure profile did not change significantly as animals aged, nor did they change in response to AG treatment.

Figure 3 shows the effects of aging and AG on the basic hemodynamic data, including basal heart rate (*HR*), cardiac output (*CO*), stroke volume (*SV*), and total peripheral resistance (R_p). Basal heart rate (Figure 3a), cardiac output (Figure 3b), and stroke volume (Figure 3c) were decreased with age ($P<0.05$); however, these decreases were not significantly modified by treatment with AG. Total peripheral resistance (Figure 3d) was increased markedly in the control group from 6 to 24 month ($P<0.05$). Although producing no alterations in *CO* (Figure 3b) and P_m (Table 1), AG exerted a significant drop of 21.9% in R_p when a comparison was made between the 24-month-old controls and the 24-month-old treated rats.

Figures 4 and 5 show the effects of aging and AG on the pulsatile nature of blood flows in arteries in terms of aortic characteristic impedance (Z_c), wave transit time (τ), and wave reflection factor (R_f). The aortic characteristic impedance (Figure 4a) was increased markedly in the 24-month-old rats as compared with the 6-month-old rats ($P<0.01$). AG administered to rats from 18 to 24 months produced a significant fall in Z_c from $0.0206\pm0.0027\,mmHg\,min\,kg\,ml^{-1}$ in the 24-month-old controls to $0.0154\pm0.0013\,mmHg\,min\,kg\,ml^{-1}$ in the 24-month-old treated rats, $P<0.05$. Meanwhile, the aortic compliances corresponding to systolic (C_s in Figure 4b), diastolic (C_d in Figure 4c), as well as mean aortic pressure (C_m in Figure 4c) were significantly decreased with age ($P<0.01$), and these decreases were prevented by administration of AG to rats for 6 month ($P<0.05$).

Magnitude of the forward pressure (P_f in Figure 5a), but not the backward pressure (P_b in Figure 5b), was significantly decreased in the 24-month-old rats as compared with the 6-month-old rats ($P<0.05$). Treatment with AG from 18 to 24 months showed an increase in P_f in the absence of any

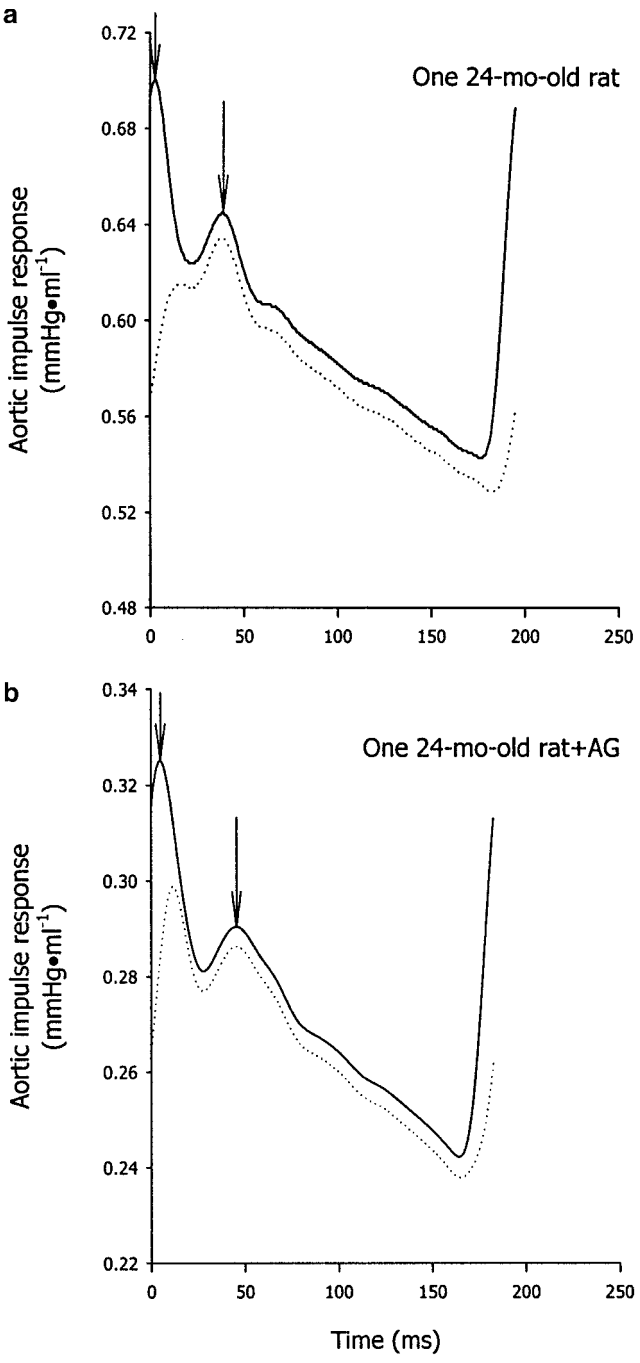

Figure 2 Impulse response function curve derived from the filtered aortic input impedance spectra shown in Figure 1. The dashed line indicates that the characteristic impedance component of the vascular impulse response has been removed. The long arrow shows the discrete reflection peak from the body circulation and the short arrow demonstrates the initial peak as a reference. In the solid line, one-half of the time difference between the appearance of the reflected peak (long arrow) and the initial peak (short arrow) approximates the wave transit time in the lower body circulation. The rat treated with AG from 18 to 24 months had increased wave transit time (b) compared with that of the untreated aged rat (a).

significant changes in P_b, leading to a decline in wave reflection factor (R_f in Figure 5c) from 0.554 ± 0.027 in the 24-month-old controls to 0.375 ± 0.030 in the 24-month-old treated rats, $P<0.01$. On the other hand, the wave transit time (τ in Figure 5d) was significantly decreased in the control group from 6 to 24 months ($P<0.01$). Administration of AG to rats for 6 months produced an increase in τ from 17.76 ± 0.43 ms in the control 24-month-old rats to 22.74 ± 0.46 ms in the 24-month-old treated rats, $P<0.01$. Both the increased τ and the decreased R_f suggest that AG may prevent the age-related augmentation in the systolic loading condition for the left ventricle coupled to the arterial system. Both the increased τ and the decreased Z_c indicate that AG has the potential to retard the age-related decline in aortic distensibility in Fisher 344 rats.

Discussion

Some studies (Cernadas *et al*., 1998; Tabernero *et al*., 2000), but not all (Challah *et al*., 1997), report that inducible nitric oxide synthase (iNOS) expression and activity in the arterial media increase with age and that this increase is inhibited by AG (Tabernero *et al*., 2000). The inhibition of NO production by AG would result in an increase in arterial blood pressure (Tabernero *et al*., 2000) as well as aortic characteristic impedance (Gaballa *et al*., 1999). However, such hypertensive effects of AG were not detected in this report when AG was administered to rats from 18 to 24 months (Table 1 and Figure 4a). The same results were reported by Li *et al*. (1996) who found that chronic treatment with AG did not significantly increase but rather decreased aortic pressure in aging rats. Corman *et al*. (1998) also reported that administration of AG caused a fall in aortic characteristic impedance and a rise in carotid distensibility in old animals. However, having no measurements on iNOS expression, we cannot reach any direct conclusions that AG has no effect to inhibit iNOS isoform activity in aged vasculature.

In this report, total peripheral resistance (R_p) was significantly increased in the 24-month-old rats as compared with the 6-month-old rats. This elevation was prevented by AG, as manifested by the reduction of 21.9% in R_p (Figure 3d). Our result was in accordance with that of the other report proposed by Corman *et al*. (1998). It has been shown that an increase in AGEs production and accumulation occurs in old animals, leading to vasodilatory dysfunction and arterial stiffening (Schnider & Kohn, 1980; Li *et al*., 1996; Corman *et al*., 1998). AGEs are reported to induce free-radical production and deplete NO concentrations, leading to a state of oxidative stress (McCance *et al*., 1993). The ability of AGEs to quench NO may diminish the vasodilatory capacity of peripheral muscular arteries in aging rats (Li *et al*., 1996). Therefore, an increase in AGEs accumulation in rats with age may be responsible for the increased vascular smooth muscle tone. The prevention of age-related vasodilatory dysfunction may result from inhibition of the AGEs formation by AG to reserve NO production in the resistance vessels (Tilton *et al*., 1993).

As for the pulsatile components of the arterial load, the aortic characteristic impedance increased (Z_c in Figure 4a) and the wave transit time decreased (τ in Figure 5d) in the control group from 6 to 24 months. Being relatively independent to body shape, the age-related change in τ could be due to the change with age in pulse wave velocity reported by Cantini *et al*. (2001). Both the augmented Z_c and the shortened τ suggest that a decline in aortic distensibility may occur in old animals. It has been shown that accumulation of AGEs is associated with changes in the biomechanical properties of

Table 1 Effects of aging and AG on body weight, LVW and aortic pressure profile in Fisher 344r rats

	BW (g)	*LVW* (mg)	P_s (mmHg)	P_d (mmHg)	P_m (mmHg)
Age (months)					
6 ($n=9$)	356.7±9.8	602.2±10.3	130.2±2.6	94.2±3.5	110.8±2.8
18 ($n=9$)	415.6±7.5	690.3±11.0	116.2±3.2	79.6±4.5	97.7±4.1
24 ($n=9$)	407.3±8.6	728.5±15.7	126.4±5.5	89.3±6.0	106.9±6.3
24+AG ($n=9$)	393.3±7.1	613.6±12.5	116.6±2.8	79.0±3.4	96.5±3.0
P value					
6 vs 18	<0.05	<0.05	NS	NS	NS
6 vs 24	<0.05	<0.05	NS	NS	NS
18 vs 24	NS	NS	NS	NS	NS
24 vs 24+AG	NS	<0.05	NS	NS	NS

All values are expressed as means±s.e. BW, body weight; LVW, left ventricular weight; P_s, systolic aortic pressure; P_d, diastolic aortic pressure; P_m, mean aortic pressure; AG, aminoguanidine.

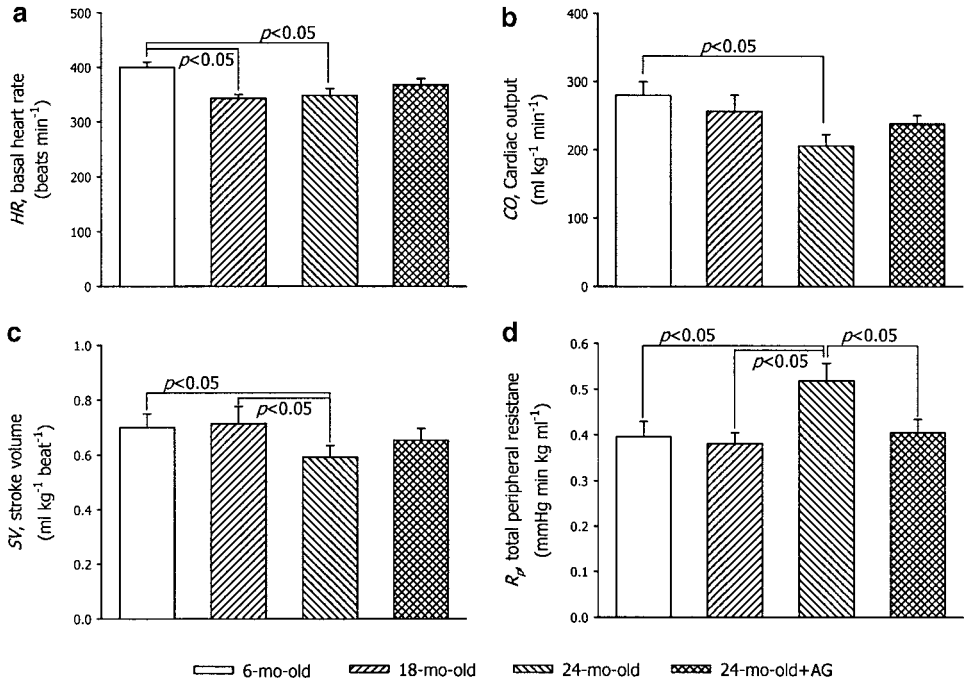

Figure 3 Effects of aging and AG on basal heart rate (*HR* in a), cardiac output (*CO* in b), stroke volume (*SV* in c), and total peripheral resistance (R_p in d). *HR*, *CO*, and *SV* were decreased with age; however, these decreases were not significantly modified by treatment with AG. By contrast, the age-related increase in R_p was prevented by administration of AG to rats from 18 to 24 months.

collagen characterized by increasing stiffness of the elastic arteries (Andreassen & Oxlund, 1985; Brownlee *et al*., 1986; Reiser, 1991; Edelstein & Brownlee, 1992; Vlassara *et al*., 1994). Therefore, an increase in AGEs accumulation in the Windkessel vessels may be responsible for the increased aortic stiffness in rats with age. Treatment with AG for 6 months prevented the age-related fall in aortic distensibility in Fisher 344 rats, as evidenced by the reduction of 25.2% in Z_c and the increase of 28.1% in τ. Such prevention of the age-related aortic stiffness by AG may result from inhibition of the AGEs-induced chemical crosslinks in the wall of the elastic reservoir. The same results were reported by Li *et al*. (1996) and Corman *et al*. (1998) who found that in the absence of any significant changes in collagen and elastin content, the prevention of arterial stiffening by AG was attributed to a decrease in the AGEs-induced crosslinking of the extracellular matrix. However, no measurements on the aortic wall histological structure were made in this report, the inferences that AG exerts its effects by inhibition of the collagen AGEs accumulation are indirect.

Changes with age in timing and magnitude of the pulse wave reflection do impair the loading condition for the left ventricle coupled to the arterial system (O'Rourke *et al*., 1987). As mentioned earlier, a reduction in τ was detected in rats with age, suggesting that the aging process may cause an early return of the pulse wave reflection from the peripheral circulation. Administration of AG from 18 to 24 months prevented this early return of the pulse wave reflection in old animals, as evidenced by the increase in τ. Meanwhile, aging contributed to a significant fall in magnitude of the forward pressure (P_f in Figure 5a), whereas the magnitude of the backward pressure remained unchanged (P_b in Figure 5b). The

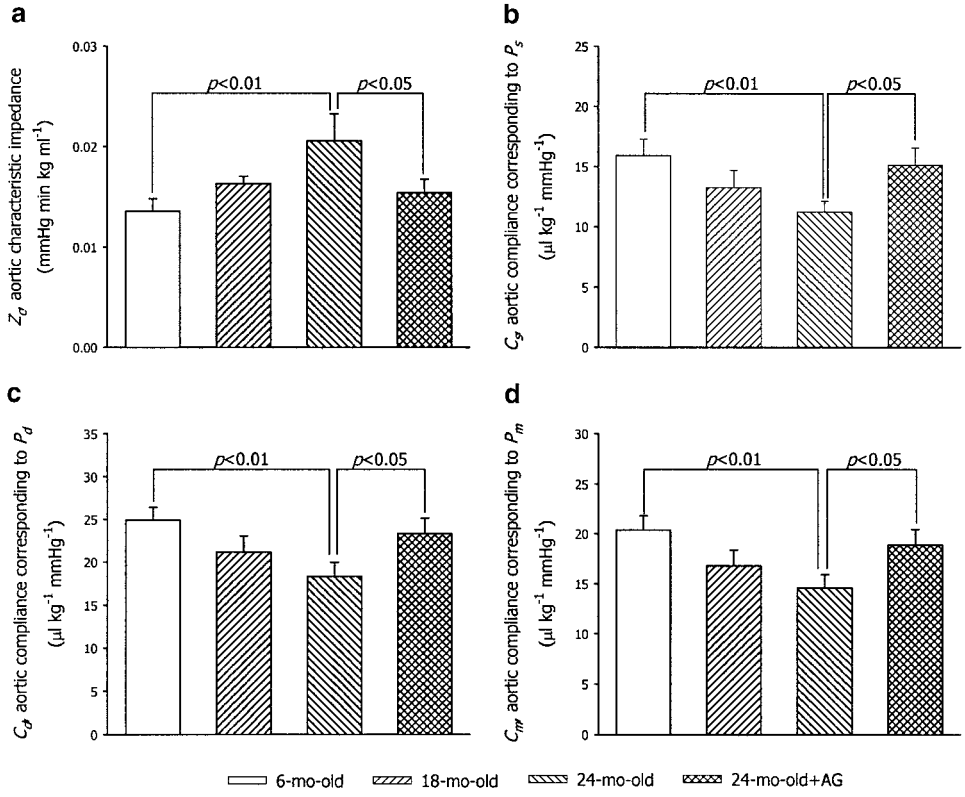

Figure 4 Effects of aging and AG on aortic characteristic impedance (Z_c in a), and systemic arterial compliances corresponding to systolic (C_s in b), diastolic (C_d in c), and mean aortic pressure (C_m in d) respectively. Z_c was significantly increased with age, and this increase was retarded by treatment with AG from 18 to 24 months. In addition, AG prevented the age-related decreases in C_s, C_d, and C_m in Fisher 344 rats.

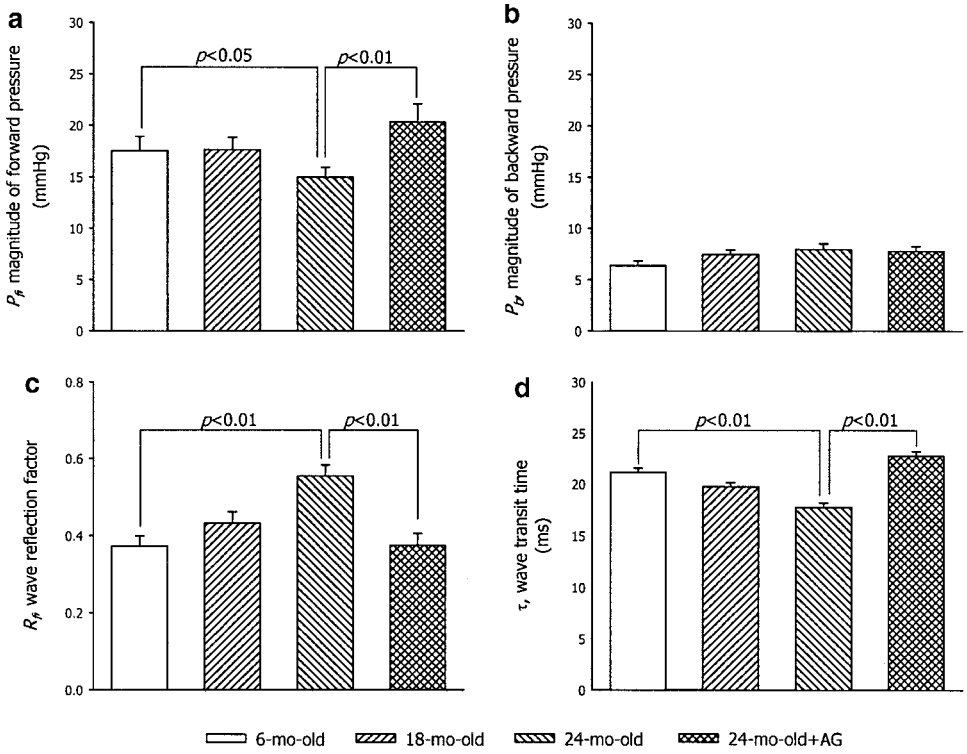

Figure 5 Effects of aging and AG on magnitude of the forward pressure (P_f in a), magnitude of the backward pressure (P_b in b), wave reflection factor (R_f in c), and wave transit time (τ in d). P_f but not P_b was significantly decreased in old animals, leading to an increase with age in R_f. This increase in R_f was prevented by administration of AG to rats from 18 to 24 months. Meanwhile, AG prevented the age-related decline in τ in Fisher 344 rats.

reduced P_f associated with the unaltered P_b was responsible for the increase in wave reflection factor in rats with age (R_f in Figure 5c). Without affecting P_b, treatment with AG for 6 months prevented the age-related fall in P_f so that the heavy reflection intensity in the arterial system was lowered, as manifested by the reduction of 32.3% in R_f. Both the increased τ and the decreased R_f suggest that AG may prevent the age-related increase in systolic load of the left ventricle. It is very likely that the prevention of age-related cardiac hypertrophy by AG treatment (Table 1) corresponds to the drug-induced decline in LV afterload.

Some limitations of the current study deserve consideration. Since the aortic input impedance cannot be measured in conscious animals, it is difficult to evaluate the effects of pentobarbital anesthesia on rats at different ages and on rats treated with AG. In this report, the results pertained only to measurements made in the open-chest rat with anesthesia. This setting induced a fall in blood pressure and may introduce reflex effects not found in the closed-chest setting. Just how much anesthesia and thoractomy affect the pulsatile hemodynamics in rats was uncertain. However, studies with other animal models suggest that the effects are small relative to the biological and experimental variability between animals (Cox, 1974).

Taken together, no hypertensive effects of AG were detected in this report when AG was administered to rats from 18 to 24 months. With an unchanged aortic pressure profile, both the decreased aortic characteristic impedance and the increased wave transit time suggest that AG may prevent the age-related decline in aortic distensibility. Meanwhile, both the increased wave transit time and the decreased wave reflection factor indicate that AG can retard the age-related augmentation in systolic loading condition for the left ventricle coupled to the arterial system. In conclusion, our data suggest that long-term treatment with AG may impart significant protection against aortic stiffening and cardiac hypertrophy in aged Fisher 344 rats.

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