Aminoguanidine and Aortic Wall Mechanics, Structure, and Composition in Aged Rats

Caroline Cantini, Pascal Kieffer, Bruno Corman, Patrick Limiñana, Jeffrey Atkinson, Isabelle Lartaud-Idjouadiene

Abstract—With aging, the aortic wall becomes stiffer. This could be because of changes in wall stress or composition. We investigated whether a specific change in wall composition, ie, accumulation of advanced glycation end products (AGEs) on the extracellular matrix, is a major factor. We measured aortic mechanics, geometry, and composition in 3-, 10-, 15-, 20-, and 30-month-old inbred normotensive Wistar-Glaxo/Rijswijk rats and in a group of 30-month-old rats treated from 20 months onward with aminoguanidine (AG, 42 mg/kg per day), an inhibitor of AGE formation. Thoracoabdominal aortic (pressure) pulse-wave velocity (PWV) increased progressively with age (44% from 3 to 30 months). This age-related increase in aortic PWV was not related to changes in wall stress. For all ages, central (and peripheral) aortic mean blood pressures were not statistically different. Dilatation occurred (18% increase in internal diameter from 3 to 30 months), but this was accompanied by outward hypertrophic remodeling, with an increase in the medial cross-sectional area of 95% and in the ratio of medial thickness to internal diameter of 29%. Wall stress decreased with age (−34%). There was an increase in the ratio of elastic modulus (calculated from the Moens-Korteweg equation) to wall stress (calculated from the Lamé equation, 117% from 3 to 30 months), suggesting that a change in the composition of the wall is responsible for the age-linked increase in wall stiffness. Dry weight decreased slightly but significantly (−14%) with age. Total protein, elastin, collagen, and nonscleroprotein protein [total − (elastin + collagen)] contents did not change with age, but calculated densities of all 4 were halved (as the medial cross-sectional area doubled). The elastin/collagen ratio was statistically similar at all ages. The only significant effect of AG treatment was a fall in PWV (−20%), leading to a fall in the elastic modulus/wall stress ratio (−27% at 10 months of AG treatment versus 30 months of no treatment). In conclusion, the age-related increase in aortic wall stiffness is prevented by 10 months of treatment with AG, which has no effect on wall stress or composition, suggesting that AG may improve aortic wall stiffness by lowering the degree of AGE-induced cross-linking of the extracellular matrix scleroproteins, such as collagen. (Hypertension. 2001;38:943-948.)

Key Words: age ■ collagen ■ rats, inbred strains ■ aorta

The wall of the large-diameter elastic arteries becomes progressively stiffer with age, and this leads to a decrease in arterial compliance and an increase in the pulsatile element of pressure.1 There is increasing evidence that the latter has harmful effects on the heart and on tissue perfusion, eg, the cerebral circulation.2,3 At the present time, the only effective therapy available to diminish wall stiffness is to reduce wall stress by lowering intravascular distending pressure with antihypertensive drugs. In some clinical situations, this is not satisfactory, and work is in progress on more specific drug targets, such as the modulation of local smooth muscle cell contraction or of the composition of the wall.

Concerning this latter approach, several recent experiments suggest that one major factor is the increase in the degree of cross-linking of scleroproteins, such as collagen, after the accumulation of advanced glycation end products (AGEs) in the wall. We tested this hypothesis by measuring aortic mechanics, geometry, and composition in 3- to 30-month-old normotensive (control) rats and in a group of 30-month-old rats treated with aminoguanidine (AG), an inhibitor of AGE formation, from 20 months onward (30-month-AG rats).

Methods

Animals
Experiments were performed in 48 normotensive male inbred Wistar-Glaxo/Rijswijk (WAG/Rij) rats. They were born in the specific pathogen-free animal facility of the Center d’Etudes de Saclay, Gif-sur-Yvette, France, and were given a commercial diet (AO4, UAR) and water ad libitum until they were 3, 10, 15, 20, or 30 months old (n=9, 6, 9, 7, and 10, respectively). A final group of rats (30-month-AG rats, n=7) was given AG hemisulfate in the drinking water (1 g/L) from 20 to 30 months of age. Because water consumption remained constant over the treatment period (16±0.5
mL per rat per day, checked once a week), the dose of AG was 42 mg/kg per day. At the end of the treatment period, animals were transferred to Nancy, where experiments were performed in accordance with the guidelines of the French Ministry of Agriculture (Laboratoire de Pharmacologie Cardiovasculaire, EA 3116, permits 54-5 and 03575).

### Aortic Blood Pressure and Pulse-Wave Velocity in Anesthetized Rats

Procedures have been described in detail elsewhere.4,5 Briefly, polyethylene cannulas were introduced, with animals under pentobarbital anesthesia (60 mg/kg), into the descending thoracic and the abdominal aorta for measurement of central and peripheral aortic blood pressures. Cannulas were filled with heparinized (5 IU/mL), gas-free 0.15 mol/L NaCl and connected to low-volume pressure transducers. The dynamic frequency response of the pressure recording system is flat with a phase lag ≤ 6° up to 30 Hz and then slightly underdamped.4

After a 30-minute habituation period, baseline parameters were averaged over periods of 4 seconds recorded every 30 seconds for 30 minutes at a sampling rate of 256 Hz. An algorithm detected systolic and diastolic pressures and calculated the mean pressure from the waveform area, the thoracoabdominal pulse amplification as peripheral aortic pulse pressure/central aortic pulse pressure, and heart rate. Thoracoabdominal pulse wave velocity (PWV, cm/s) was calculated as the distance between the 2 cannula tips divided by the transit time (in milliseconds), measured by an algorithm that systematically shifted in time the peripheral pressure waveform with respect to the central pressure waveform and determined the value of the time shift, giving the highest correlation between the 2 waveforms.4,5

### Plasma Glucose Concentration, Thoracic Aorta Geometry, Wall Stress, and Elastic Modulus

At the end of the hemodynamic measurements, 1 mL of blood was collected for plasma glucose determination by the glucose oxidase method.

Animals were then euthanized with a sodium pentobarbital overdose. The aortic tree was perfused for 30 minutes at the baseline central aortic mean blood pressure (CAMBP) with 10% (vol/vol) formalin containing PBS. This procedure holds the diameters at the distension corresponding to CAMBP, assuming that shrinkage of tissues is similar in all groups. A 0.5-cm sample of the descending thoracic aorta was excised. Three 20-μm-thick sections were stained with hematoxylin-eosin for measurement of internal diameter and media thickness by using the Saisam algorithm (Microvision Instruments). Medial cross-sectional area (MCSA, mm²) was calculated as follows: m/4(D²−D²), where Dₘ and D are outer and inner diameter (in millimeters), respectively, and Dₘ=D₋2h, where h is medial thickness (in millimeters).

Elastic modulus and wall stress, both 10⁶ dyne/cm², were calculated from the Moens-Korteweg and Lamé equations, respectively: elastic modulus = PWV² × D × h/ρ; wall stress = CAMBP × D²/2h, with D and h expressed in centimeters, and ρ indicating blood density (1.05 g/cm³).

### Thoracic Aorta Wall Composition

A second 1-cm sample of the descending thoracic aorta was weighed and dehydrated at 105°C to determine the percentage of dry weight. Total wall calcium content was determined by atomic absorption spectrophotometry after mineralization and nitric acid digestion.6

A third 0.3-cm sample of the thoracic aorta was weighed and hydrolyzed in hydrochloric acid (6 mol/L) at 105°C for 24 hours. Protein content (mg/g dry wt) was determined by the dinitrofluorobenzene reaction, with a value of 92 used for the average molecular weight of an amino acid. Collagen (mg/g dry wt) was determined by the chloramine T and para-dimethylaminobenzaldehyde reaction and expressed as hydroxyproline content × 7.46.7 Desmosine and isodesmosine cross-linking amino acids specific to elastic contents were determined by capillary zone electrophoresis,8 and elastin (mg/dry wt) was calculated as desmosine plus isodesmosine × 200.9 Estimated densities of elastin and collagen (µg · mg⁻¹ · mm⁻²) were calculated as content/MCSA, and percentages of elastin and collagen were calculated as content × 100/protein content. The elastin/collagen ratio was also calculated. Nonscleroprotein content was calculated as protein content − (elastin content + collagen content).

### Statistical Analysis

Values are given as mean±SEM. Differences between groups were evaluated by using ANOVA, followed by the Bonferroni post hoc test, focusing on the effects of age (3 to 30 months in control rats).

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**TABLE 1. Body Weight, Aortic Length, Plasma Glucose Concentration, Baseline Central and Peripheral Aortic Blood Pressures, and Thoracoabdominal Pulse Amplification in WAG/Rij Rats**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control Rats</th>
<th>3 Month (n=9)</th>
<th>10 Month (n=6)</th>
<th>15 Month (n=9)</th>
<th>20 Month (n=7)</th>
<th>30 Month (n=10)</th>
<th>30-Month-AG Rats (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td></td>
<td>298±6</td>
<td>347±7*</td>
<td>373±5*</td>
<td>386±8*</td>
<td>403±8*</td>
<td>362±15†</td>
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<td>Aortic length, cm</td>
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<td>8.8±0.3</td>
<td>9.3±0.2</td>
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<td>Plasma glucose concentration, g/L</td>
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<td>1.3±0.1</td>
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<td>1.1±0.1</td>
<td>1.2±0.1</td>
<td>1.4±0.3</td>
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<td>Central aortic blood pressures, mm Hg</td>
<td></td>
<td>131±3</td>
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<td>127±3</td>
<td>131±5</td>
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<td>106±4</td>
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<td>Pulse</td>
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<td>23±2</td>
<td>28±1</td>
<td>25±1</td>
<td>30±2</td>
<td>28±2</td>
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<tr>
<td>Peripheral aortic blood pressures, mm Hg</td>
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<tr>
<td>Pulse</td>
<td></td>
<td>1.5±0.1</td>
<td>1.6±0.1</td>
<td>1.5±0.1</td>
<td>1.4±0.1</td>
<td>1.3±0.1*</td>
<td>1.3±0.1</td>
</tr>
</tbody>
</table>

Three, 10, 15, 20, and 30 mo indicate 3-, 10-, 15-, 20-, and 30-month-old control rats, respectively. Values are mean±SEM. *P<0.05 vs 3 mo; †P<0.05 vs 30 mo.
and treatment (30-month-AG rats compared with 30-month-old control rats).

Results

Body Weight, Aortic Length, Plasma Glucose Concentration, Baseline Aortic Blood Pressures, PWV, and Pulse Amplification

Body weight and aortic length increased with age in control rats ($P<0.0001$ and $P=0.01$, respectively) (Table 1). Treatment with AG did not change growth (aortic length remained constant, $P=0.95$) but slightly decreased body weight by 10% ($P=0.02$). Plasma glucose concentration did not significantly change with age ($P=0.18$) or after treatment with AG ($P=0.57$).

Baseline central and peripheral aortic blood pressures (Table 1) did not significantly change with age ($P=0.33$ for CAMBP) (Figure 1); AG had no effect ($P=0.46$ for CAMBP). Thoracoabdominal pulse amplification decreased with age by 10% from 3 to 30 months ($P=0.03$); AG had no effect ($P=0.63$). PWV increased with age by 44% from 3 to 30 months in control rats ($P=0.0004$) and decreased by 20% in 30-month-AG versus 30-month-old control rats ($P=0.03$) (Figure 1).

Thoracic Aorta Geometry, Wall Stress, and Elastic Modulus

The thoracic aorta underwent outward hypertrophic remodeling, inasmuch as the MCSA increased by 95% from 3 to 30 months ($P<0.0001$) (Table 2), with the internal diameter increasing less (17%, $P<0.0001$) than medial thickness (60%, $P<0.0001$) or the medial thickness/internal diameter ratio (40%, $P<0.0001$). Treatment with AG had no effect on aortic geometry ($P>0.05$ for each parameter).

Because the medial thickness/internal diameter ratio increased with age but CAMBP did not change, wall stress significantly decreased (by 34%) from 3 to 30 months ($P<0.0001$) (Figure 2); AG had no effect ($P=0.39$). The elastic modulus did not significantly increase with age ($P=0.08$), but the elastic modulus/wall stress ratio increased by 117% from 3 to 30 months ($P<0.0001$) (Figure 2); AG lowered the elastic modulus by 33% and the elastic modulus/wall stress ratio by 27% ($P=0.03$ and $P=0.04$, respectively, for 30-month-AG rats compared with 30-month-old control rats).

| TABLE 2. Thoracic Aorta Wall Geometry in WAG/Rij Rats |
|---------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Parameters                      | 3 Month        | 10 Month       | 15 Month       | 20 Month       | 30 Month       | 30-Month-AG Rats |
| D_i, mm                        | 1.47±0.03      | 1.38±0.06      | 1.40±0.03      | 1.70±0.05*     | 1.73±0.06*     | 1.66±0.03      |
| h, μm                          | 62±2           | 56±3           | 54±1           | 90±5*          | 101±5*         | 98±5           |
| h/D_i, ×10^{-3}                | 42±1           | 41±2           | 42±8           | 53±2*          | 54±2*          | 59±3           |
| MCSA, mm²×10^{-3}              | 301±16         | 254±19         | 269±11         | 511±41*        | 583±46*        | 546±38         |

D_i indicates internal diameter; h, medial thickness. Values are mean±SEM. *$P<0.05$ vs 3 mo.
Thoracic Aorta Wall Composition

The dry weight of the thoracic aorta decreased slightly (by 14%), and total protein content increased slightly (by 6%) from 3 to 30 months (P=0.0006 and P=0.02, respectively) (Table 3). Total calcium content increased by 68% from 3 to 30 months (P=0.001). Collagen content, expressed either as milligram per gram dry weight or as percentage of protein content, did not change with age (P>0.05 for both); estimated collagen density significantly decreased by 45% from 3 to 30 months (P<0.0001). Age-related changes in elastin content, expressed either as milligram per gram dry weight, percentage, or density, and in the elastin/collagen ratio did not reach statistical significance (P=0.12 to 0.54). Non-scleroprotein content did not change with age (P=0.16). AG had no impact on any of these parameters (P>0.05).

Discussion

The aortic wall becomes stiffer with age in the rat, as shown by the increase in PWV. An increase in wall stress cannot explain this increase in wall stiffness, because in the present study, calculated wall stress significantly fell with age. This was because CAMBP did not significantly change and pronounced outward hypertrophic remodeling “overcompensated” for the increase in internal diameter. Changes in wall stress and stiffness are similar to those previously reported by Michel et al in the same rat strain and over a similar age range. In their study, wall stress (calculated from the data they gave) did not significantly fall but remained constant with age. Wall stiffness increased, as shown by an increase in aortic characteristic impedance and a decrease in systemic and local compliance in spite of an increase in arterial volume.

In the studies of Michel et al and Corman et al, who used the same rat strain, changes in the wall content of scleroprotein appear to be involved in the age-related changes in wall stiffness. However, interpretation may differ according to the method used to evaluate scleroprotein content. In both studies, the relative amount of collagen per cross section or longitudinal area of the aortic wall (measured by histomorphometry after Sirius red staining) increased with age, as did the absolute amount (measured by the hydroxyproline assay of the NaOH-soluble fraction). Results for elastin were less consistent: the relative amount (histomorphometry after orcein staining) increased or remained constant with age, whereas the absolute amount (hydroxyproline assay on the NaOH-insoluble fraction) decreased. Histological methods are fraught with the uncertainty as to what is actually stained: elastin, collagen, proteoglycans, or other fiber components. Wolinsky highlighted the importance of determining the absolute amounts of collagen and elastin by chemical methods instead of relative amounts “when indisputable evidence for a net change in the amount of a tissue component is desired.” However, results on changes in absolute amounts of scleroproteins with age in normotensive rats are contradictory (see Figure 3). Globally, excepting the studies of Wolinsky, changes in elastin and collagen from youth to senescence are nonexistent (Brüel and Oxlund and present study) or slight. Overall, these observations suggest that changes in the absolute amounts of scleroproteins are not a predominant factor in the age-related changes in stiffness of the aortic wall. This is confirmed in the present study by the fact that AG reduced wall stiffness in old rats, with no change in wall composition. The remaining hypotheses for the age-linked increase in wall stiffness are changes in the chemical, molecular, or supramolecular nature of the wall components or a change in the smooth muscle tone of the wall. Regarding the former, we have suggested that massive wall calcification may be an important feature of the age-linked increase in wall stiffness in humans and in certain animal models of induction.
of calcification, in which total calcium content of the aortic wall increases at least 10-fold. Michel et al. reported that calcium bound to elastin increased slightly but significantly with age. In the present study, as in a previous study performed in WAG/Rij rats, total calcium content of the aortic wall increased with age, but only 1.7-fold. Overall, these observations suggest that wall calcification is a relatively minor factor.

AG reduced aortic wall stiffness in old rats, with no change in wall stress or scleroprotein composition. This observation is in line with the hypothesis that AGE-induced cross-linking of scleroproteins in adult animals increases the aortic elastic modulus and with a previous report showing that the age-linked decrease in arterial distensibility was attenuated in diabetes. This is not the case in the present study, because AG lowered stiffness but did not lower wall water content.

In conclusion, stiffness of the aortic wall increases with age in WAG/Rij rats, although wall stress actually decreases; changes in absolute amounts of scleroproteins or calcium do not appear to be the predominant factor in this age-related stiffening. Chronic treatment with AG reduced wall stiffness in old rats, with no change in wall stress or composition, suggesting that AG improves aortic wall stiffness by lowering the degree of AGE-induced cross-linking of the extracellular matrix scleroproteins, such as collagen.

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References

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