Pioglitazone Improves Aortic Wall Elasticity in a Rat Model of Elastocalcinotic Arteriosclerosis

Virginie Gaillard, Daniel Casellas, Carole Seguin-Devaux, Hervé Schohn, Michel Dauça, Jeffrey Atkinson, Isabelle Lartaud

Abstract—Specific treatment of age-related aortic wall arteriosclerosis and stiffening is lacking. Because ligands for peroxisome proliferator–activated receptor γ have beneficial effects on the arterial wall in atherosclerosis, via an antiinflammatory mechanism, we investigated whether long-term pioglitazone (Pio) treatment protects against another form of vascular wall disease, arteriosclerosis. We evaluated, in a rat model of elastocalcinotic arteriosclerosis (hypervitaminosis D and nicotine [VDN]), whether Pio (3 mg·kg⁻¹ per day for 1.5 month PO) attenuated arteriosclerosis and its consequences: aortic wall rigidity, increased aortic pulse pressure, and left ventricular hypertrophy. In VDN rats, medial calcification was associated with monocyte/macrophage infiltration and induction of tumor necrosis factor α and interleukin 1β. Pio increased nuclear peroxisome proliferator–activated receptor γ immunostaining in the aortic wall, decreased tumor necrosis factor α (P<0.05 versus VDN Pio⁻), tended to decrease interleukin 1β mRNA expression (P=0.08 versus VDN Pio⁻), blunted aortic wall calcification (271±69, P<0.05 versus VDN Pio⁻ 562±87 μmol·g⁻¹ dry weight) and prevented fragmentation of elastic fibers (segments per 10 000 μm²: 8.4±0.3; P<0.05 versus VDN Pio⁻ 10.5±0.6). Pio reduced aortic wall stiffness (elastic modulus/wall stress: 4.8±0.6; P<0.05 versus VDN Pio⁻ 10.0±1.6), aortic pulse pressure (30±2 mm Hg; P<0.05 versus VDN Pio⁻ 39±4) and left ventricular hypertrophy (1.58±0.05 g·kg⁻¹; P<0.05 versus VDN Pio⁻ 1.76±0.06). In conclusion, long-term Pio treatment attenuates aortic wall elastocalcinosis and, thus, lowers aortic wall stiffness, aortic pulse pressure, and left ventricular hypertrophy. (Hypertension. 2005;46:372-379.)

Key Words: extracellular matrix ■ peroxisome proliferator–activated receptor ■ arteriosclerosis ■ calcium ■ pulse pressure

An age-related increase in aortic wall stiffness is an independent risk factor for cardiovascular morbidity and mortality.¹⁻³ There is no specific treatment, thus the research for drug targets is of great importance. Aortic wall stiffening arises principally from extracellular matrix remodeling of the media. Calcification and fragmentation of the elastic fiber network,⁴⁻³ and nonenzymatic cross-linking (glycation)⁶⁻⁷ of collagen fibers, are the most important determinants.

Several of these molecular mechanisms (calcification, glycation) have an inflammatory element. Thus, we investigated a new antiinflammatory pharmacological target, the peroxisome proliferator–activated receptor (PPAR)–γ, on the basis that thiazolidinediones (TZDs) specific PPAR–γ ligands have beneficial protective effects on the arterial wall in atherosclerosis⁸⁻⁹ and arteriosclerosis,¹⁰ via antiinflammatory mechanisms.

The beneficial antiproliferative and antifibrotic effects of pioglitazone (Pio) were demonstrated using a rat model of inflammation-induced wall fibrosis (long-term inhibition of endothelial NO synthesis).¹⁰ In the present study, we focused on another determinant of extracellular matrix remodeling: medial elastocalcinoses. We used an animal model of calcification and degradation of elastic fibers, the rat treated with vitamin D₃ and nicotine (VDN).¹¹⁻¹³ To our knowledge, this is the only model showing extensive aortic medial calcification, in the absence of fibrosis or any change in wall stress. In VDN rats, extracellular calcium-binding proteins and ectopic apatite deposition develop on medial elastic fibers, followed by fragmentation of the latter (elastocalcinoses).¹¹⁻¹² This causes wall stiffening, then elevation of pulse arterial pressure (with no change in mean arterial pressure) and compensatory left ventricular hypertrophy.¹²⁻¹³

Our working hypotheses were that (1) VDN-induced calcification and elastic fiber fragmentation represent, at least partially, an inflammatory response of the aortic wall; and (2) long-term treatment of VDN rats with Pio¹⁰ (Pio), by counteracting the inflammatory process,⁸⁻¹⁰ reduces calcification and elastic fiber fragmentation, therefore leading to a de-
crease in wall elastic modulus and the consequences of increased aortic wall rigidity, namely, increased pulse pressure and left ventricular hypertrophy. In a separate experiment, we checked whether the beneficial effect of Pio was attributable to a short-term, functional, or long-term structural effect on the aortic wall.

Methods

Animals and Drug Treatment

Seven-week-old male Wistar rats (Ico: Wi, IOPS AF/Han, Charles River Laboratories, L’Arbresle, France) were kept under standard conditions for 1 week before VDN treatment.

For the long-term experiment, VDN rats (n=26) were treated on day 0 (D0) with vitamin D3 (300 000 IU·kg⁻¹·IM) and nicotine (2×25 mg·kg⁻¹·IM) and distilled water (PO). From D1 to D44, rats received Pio (3 mg·kg⁻¹·day⁻¹) per day. Hemodynamic parameters were measured 2 hours later (time of the peak in the plasma concentration of Pio, high-pressure and left ventricular hypertrophy. In a separate experiment, we checked whether the beneficial effect of Pio was attributable to a short-term, functional, or long-term structural effect on the aortic wall.

Aortic Blood Pressure and Pulse Wave Velocity

On D45, polyethylene cannula, connected to low-volume pressure transducers, were introduced into the descending thoracic and abdominal aorta for measurement of baseline central and peripheral blood pressures.Δ,12–15 An algorithm detected systolic and diastolic blood pressures.6,12–15 Pulse wave velocity (PWV) was calculated as the distance between the 2 cannula tips (measured in situ following euthanasia) divided by the pulse wave transit time.6,12–15 Pulse wave velocity (PWV) was calculated as the distance between the 2 cannula tips (measured in situ following euthanasia) divided by the pulse wave transit time.

Figure 2. A, Total calcium content of the thoracic aortic wall in VDN and non-VDN rats, treated or not with Pio for 44 days. *P<0.05 vs non-VDN rats, †P<0.05 vs Pio⁻. PVDN<10⁻⁴; Pnon-VDN=0.036. B, Light microscopic views of the aortic wall in a VDN rat. B1 to B3, Successive sections of the same aorta. Calcium was revealed by the Von Kossa stain (black deposits); monocyte/macrophages were immunostained with a monoclonal mouse anti-rat ED-1 (red deposits). B1 and B2, Intense calcium deposition along the elastic lamellae. Monocyte/macrophages are present between the calcified lamellae (black arrow), within the adventitia (black arrowheads), and at the surface of the endothelium (black star). B3, Sparse calcium deposits (white arrow) and monocyte/macrophage infiltration within the adventitia (black arrowhead). The anti-ED-1-antibody was omitted in the adjacent aortic section, and B4 depicts the area shown in B1. Bar indicates 100 micrometers (applies to all panels); L, lumen.

Figure 1. Western blot analysis of PPAR-γ protein in VDN and non-VDN rats, treated or not with Pio for 44 days. A, Representative examples of the bands obtained. B, Densitometric quantification. *P<0.05 vs non-VDN. PVDN=0.050, PPio=0.512, PVDN/Pio=0.299.
the harmonic composition of the pulse pressure signal. This would alter PWV measured, as it did here (by the foot-to-foot method). However, changes in heart rate were relatively minor (see Results).

Plasma Glucose Concentration, Left Ventricular Weight, Thoracic Aorta Geometry and Mechanics, and Wall Elastic Network

One milliliter of arterial blood was collected for determination of plasma glucose (glucose oxidase method). After euthanasia (sodium pentobarbital overdose), the left ventricle, including septum, was dissected free and weighed; left ventricular hypertrophy was estimated as left ventricle/body weight. The apex of the ventricle (150 mg) was dehydrated at 110°C to determine the percentage of dry weight.

The first centimeter of the descending thoracic aorta (location in study by Niederhoffer et al.22) was removed, fixed in formaldehyde (10% in phosphate buffered saline), and embedded in paraffin for histomorphometric analyses and evaluation of monocyte/macrophage infiltration (see above). All tests were performed by 2 independent, blinded observers. The internal diameter (Di) and overall thickness (h) were measured on 20-μm thick sections stained with hematoxylin-eosin (Saisam algorithm; Microvision Instruments, Evry, France).

Elastic modulus (EM = PWV² · D/h; Moens-Korteweg) and wall stress (WS = central aortic mean blood pressure · D/2 · h; Lamé) were calculated with ρ·blood density, 1.05 g · cm⁻³. EM/WS was used as an isotropic index of intracranial aortic wall stiffness.6,15

Fragmentation of the medial elastic fiber network (excluding the external and internal laminae) was evaluated on 10-μm thick sections stained with Weigert solution by measuring the increase in the number15 and the decrease in the length of elastic lamellae.

Monocyte/Macrophage Infiltration in the Aortic Wall, Interferon γ, Tumor Necrosis Factor α, and Interleukin 1β mRNA Expression

Aortic calcium was visualized as black deposits on deparaffinized/rehydrated sections using the von Kossa stain.11,16 Sections were then antigen-retrieved in citrate buffer (pH 6.40 minutes, 97°C then 20 minutes, room temperature). Monocyte/macrophages were immunostained by overnight exposure (4°C) to a monoclonal mouse anti-ED-1 antibody (dilution 1:500; mca341r, Serotec, UK). The antibody was revealed as red deposits using an indirect streptavidin-biotin method with H2O2/3-amino-9-ethylcarbazole as chromogen (DAKO Chemmate Detection Kit; peroxidase).

Total mRNA was extracted from the second centimeter12 of the descending thoracic aorta (6 to 9 rats per group) using Tri-Reagent (Qiagen, Courtaboeuf, France). Total mRNA concentration was measured, its integrity was confirmed, and reverse transcription–polymerase chain reaction (PCR) and PCR were performed as previously described17,18 (using the housekeeping gene L27: amplimers 5′-TCTTGCCGTGACCGCTACTC-3′, sense; and 5′-CCACAGAGTACCTTGTGGGC-3′, antisense; annealing temperature 62°C. PCR product 225 bp). The PCR products (separated on a 2% agarose gel containing 0.5 μg ml⁻¹ ethidium bromide) were quantified as density ratio (PCR product of interest/L27) by densitometry (GelDoc; Bio-Rad). Each aorta was analyzed in triplicate as described,21 using a polyclonal rabbit PPAR-γ antisera22 (dilution 1:1500) then fluorescein-conjugated goat anti-rabbit IgG (1:50); UV microscopy).22 The analysis was performed on 3 to 4 rats per group, 3 aortic sections per rat, focusing on 30 to 40 medial smooth muscle cells per section, to evaluate the percentage of smooth muscle cells showing nuclear PPAR-γ staining.

Statistics

Parametric values (means±SEM) were analyzed by a 2-way ANOVA (PVDN, Phe, Pdio, PVDN·Pio) plus the Bonferroni post hoc test and nonparametric values (immunostaining) by the Mann–Whitney U test. P<0.05 vs non-VDN Pio †P<0.05 vs Pio.
Vitamin D3 (Duphar D3) was purchased from Duphar B.V. (Weesp, The Netherlands), sodium pentobarbital from Sanofi Santé Nutrition Animale (Libourne, France), and other chemicals from Sigma Chemical Co (St Louis, Mo). Pio was a gift of Takeda Chemicals Industries Ltd (Osaka, Japan).

**Results**

**PPAR-γ, Monocyte/Macrophage Infiltration, Cytokine Expression, Elastic Network, Composition of the Aortic Wall, and Thoracic Aorta Geometry**

VDN treatment increased PPAR-γ protein content (1.6×), but long-term treatment with Pio had no effect (Western blot, Figure 1). Medial smooth muscle cells presented positive cytoplasmic immunostaining for PPAR-γ, with no difference between non-VDN and VDN. Pio⁺ increased nuclear PPAR-γ staining to 95±2% (non-VDN Pio⁻ + VDN Pio⁺ rats, P<0.05 versus 65±10% in non-VDN Pio⁻ + VDN Pio⁻ rats).

The calcium content of the aortic wall increased 43× in VDN rats (Figure 2); Pio⁺ partially prevented calcification (VDN Pio⁺: 21× versus non-VDN rats). In VDN rats, the media exhibited heavy calcium deposits along the elastic lamellae and monocyte/macrophage infiltration between calcified lamellae (Figure 2). In VDN Pio⁺, the occurrence of calcified lesions along the aortic segment was lower, but, when present, lesions were similar in nature to those observed in VDN Pio⁻ and showed monocyte/macrophage infiltration.

**Table 1. Thoracic Aorta Geometry, Elastic Network, and Composition of the Aortic Wall of VDN and Non-VDN Rats Treated or Not With Pio for 44 Days**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Non-VDN</th>
<th>VDN</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aortic geometry</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dₐ, mm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pio⁻</td>
<td>1.85±0.06</td>
<td>2.28±0.10</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td>Pio⁺</td>
<td>1.89±0.04</td>
<td>1.96±0.06</td>
<td></td>
</tr>
<tr>
<td>Medial thickness, μm</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pio⁻</td>
<td>72±3</td>
<td>78±2</td>
<td>P&lt;0.014</td>
</tr>
<tr>
<td>Pio⁺</td>
<td>72±2</td>
<td>74±2</td>
<td></td>
</tr>
<tr>
<td>Elastic fiber fragmentation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of medial elastic segments per 10 000 μm²</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pio⁻</td>
<td>8.0±0.3</td>
<td>10.5±0.6</td>
<td>P&lt;10⁻⁴</td>
</tr>
<tr>
<td>Pio⁺</td>
<td>7.6±0.3</td>
<td>8.4±0.3</td>
<td></td>
</tr>
<tr>
<td>Ratio of the length of elastic fibers to the length of the medial area section</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pio⁻</td>
<td>0.95±0.03</td>
<td>0.61±0.04</td>
<td>P&lt;10⁻⁴</td>
</tr>
<tr>
<td>Pio⁺</td>
<td>1.00±0.02</td>
<td>0.88±0.03</td>
<td></td>
</tr>
<tr>
<td>Aortic wall composition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry weight/wet weight, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pio⁻</td>
<td>42±1</td>
<td>46±2</td>
<td>P&lt;0.312</td>
</tr>
<tr>
<td>Pio⁺</td>
<td>44±1</td>
<td>43±2</td>
<td></td>
</tr>
<tr>
<td>Total protein content, mg·g⁻¹ wet weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pio⁻</td>
<td>322±13</td>
<td>268±16</td>
<td>P&lt;0.025</td>
</tr>
<tr>
<td>Pio⁺</td>
<td>313±11</td>
<td>299±13</td>
<td></td>
</tr>
<tr>
<td>Collagen content, mg·g⁻¹ wet weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pio⁻</td>
<td>135±9</td>
<td>123±8</td>
<td>P&lt;0.298</td>
</tr>
<tr>
<td>Pio⁺</td>
<td>134±4</td>
<td>128±5</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.05 vs non-VDN rats, †P<0.05 vs Pio⁻.
Interferon γ mRNA content did not change in VDN rats (results not shown), but tumor necrosis factor (TNF)-α and interleukin (IL)-1β mRNA expression increased in parallel with the degree of calcification (Figure 3). There was a significant positive relationship between IL-1β expression and calcium content of the aortic wall in VDN Pio− rats (slope \( \times 10^{-4} \)=9.8±1.7 μmol\(^{-1}\)·g; \( r=0.9035; P<0.05; \) intercept=0.25±0.11; \( n=9 \)) but not between TNF-α and calcium content. Pio+ decreased TNF-α (\( P<0.05 \) versus VDN Pio−) and tended to decrease IL-1β (\( P=0.08 \)) expression by 38% and 25%, respectively. In VDN Pio+, the correlation between IL-1β and calcium content of the wall was not statistically significant.

The medial elastic network showed severe fragmentation and disorganization in VDN rats (Figure 4), with an increase in the number (+31%) and a decrease in the length (−36%) of lamellae (Table 1). Pio− blunted elastic network fragmentation in VDN rats.

The dry weight and collagen content of the aortic wall were similar in all groups (Table 1). Protein content decreased in VDN rats (−17%); Pio+ had no effect.

Internal diameter increased by 22% (\( P<0.05 \)) in VDN rats, with no change in medial thickness (Table 1). Chronic Pio treatment prevented dilatation in VDN rats but had no effect on medial thickness.

**Aortic Blood Pressure and Wall Mechanics**

PWV (\( \times 1.5 \), Table 2) and EM/WS (\( \times 2.8 \), Figure 5) increased in VDN rats, with no change in wall stress (Table 2). Pio− decreased PWV and EM/WS in VDN rats only (\( P_{\text{VDN:Pio}}<0.05 \)) to values that were not significantly different from those of non-VDN Pio− rats (PWV, \( P=0.150 \); EM/WS, \( P=0.189 \)).

There was a significant positive relationship between EM/WS and calcium content of the aortic wall in VDN rats (slope =0.0074±0.0015 μmol\(^{-1}\)·g; \( r=0.719; P<0.05; \) intercept=3.44±0.77; \( n=26 \)) but not in non-VDN rats. The linear regression slope for VDN Pio− rats (0.0061±0.0017 μmol\(^{-1}\)·g; \( r=0.697; P<0.05; \) intercept=3.65±0.73; \( n=17 \)) was lower (\( P=0.0018 \)) than that for VDN Pio+ rats (0.0093±0.0041 μmol\(^{-1}\)·g; \( r=0.678; P=0.05; \) intercept=2.69±2.59; \( n=9 \)).

Central aortic mean blood pressure was similar in all groups (Table 2); pulse pressure increased by 56% in VDN rats (Figure 4). Pio lowered aortic pulse pressure (Figure 4) to a value not significantly different from that of non-VDN Pio− rats (\( P=0.083 \)).

In the short-term experiment, VDN Pio− rats showed calcification and stiffening (calcium content 46×; PWV 1.5× non-VDN) of the aortic wall; pulse pressure increased by 61% versus non-VDN, with no change in mean aortic blood pressure or heart rate. Acute Pio administration had no effect on aortic wall calcium content, PWV or pulse pressure; it slightly increased mean aortic pressure (+4%) and decreased heart rate (−10%) both in VDN and non-VDN rats (\( P<0.05 \)).

**Body Weight, Plasma Glucose Concentration, and Cardiac Parameters**

VDN rats lost weight between \( D_0 \) and \( D_6 \) (−18%, results not shown), then recovered normal growth. At \( D_{6\alpha} \), body weight was 5% lower in VDN than in non-VDN rats (Table 3). Pio+ increased body weight in both VDN and non-VDN rats (+10%). Plasma glucose concentrations were similar in all groups (Table 3).

Heart rate, left ventricle weight, and the percentage of myocardium dry weight were similar in all groups (Table 3). Left ventricle/body weight increased by 16% in VDN rats (Figure 5); Pio lowered this ratio to a value not significantly different from that of non-VDN Pio− rats (\( P=0.150 \)).
Gaillard et al Pioglitazone Protects Against Elastocalcinosis 377

Discussion

VDN treatment reveals an inflammatory response of the aortic wall (monocyte/macrophage infiltration and induction of proinflammatory cytokines) that accompanies calcification associated with medial elastic fiber fragmentation and wall stiffening. Treatment of VDN rats with Pio, which purportedly acts as an antiinflammatory agent in the arterial wall,8–10,23 blunts elastocalcinosis and its structural and hemodynamic consequences. Whether this is attributable to an antiinflammatory effect is discussed below.

The beneficial effect of Pio on vascular structure and hemodynamics was attributable not to a short-term but to a long-term effect; with short-term administration, Pio had no effect on aortic wall calcium content, PWV, or pulse pressure.

The anticalcinotic effect of Pio in VDN is more likely to be caused by activation of PPAR-γ rather than to a change in PPAR-γ expression. Pio treatment did not induce any change in the PPAR-γ protein content in VDN, and the decrease in aortic wall calcium content did not correlate with changes in PPAR-γ levels. VDN treatment itself causes a significant increase in PPAR-γ (30% to 40%). Thus, although Pio tended to increase PPAR-γ in non-VDN rats, it does not produce any further significant increase in VDN rats. This does not exclude that nuclear activation of PPAR-γ by Pio may induce expression of target genes involved in the anticalcinotic effect. Another possibility is that PPAR-γ expression increases at an early stage of Pio treatment in VDN rats (and produces an anticalcinotic effect) and that later (D45) the anticalcinotic effect remains, but the difference in PPAR-γ expression is masked.

An antiinflammatory action of Pio (witnessed by the decreased expression in TNF-α and IL-1β) is suggested in our VDN model but may be less important or more subtle than in other articles.8–10,23 In VDN rats, aortic wall calcification was strongly associated with monocyte/macrophage infiltration and increased expression of TNF-α and IL-1β; IFN-γ mRNA, which is produced by lymphocytes and/or natural killer cells, did not change. Pio did not modify the strong association between calcium deposits and monocyte/macrophage infiltration because, when present, aortic calcified lesions were similar in nature to those observed in VDN Pio+; however, the frequency of calcified lesions along the aortic segment was lower in VDN Pio+. Moreover, the lack of correlation between IL-1β mRNA and calcium contents in VDN Pio+ suggests that the antiinflammatory effect of Pio is not the only mechanism responsible for the decrease in aortic wall calcification in the VDN model.

Other hypotheses can be evoked. Pio may attenuate the recruitment of cells producing proteins involved in arterial wall calcification. Firstly, in the VDN model, arterial wall calcification is initiated by recruitment of S-100 cells and deposition of extracellular S-100 calcium-binding proteins.12 Secondly, Pio and vitamin D are ligands for nuclear receptors, which may interfere with each other,24 and those modify bone calcium metabolism and at a later stage arterial wall calcification. In VDN, part of the arterial ectopic apatite deposited is of bone origin. The calcium content of the femoral bone decreases during the first 2 weeks following treatment;45 inhibition of osteoclasts prevents arterial calcification in vitamin D–treated rats,26 and TZDs inhibit bone resorption induced by vitamin D.27 Thirdly, part of the calcium deposited on the wall elastic fibers is released from smooth muscle cells following calcium overload-induced necrosis28 produced by a direct or indirect (sympathomimetic) action28 of nicotine. Calcium channel blockers such as isradipine reduce aortic wall calcification in the VDN model,4 and TZDs block smooth muscle cell calcium channels.29

Treatment with Pio halved calcium deposition, which remained, none-the-less, elevated (21-fold above non-VDN rats). In spite of this, Pio normalized EM/WS (Figure 4) in VDN rats. This suggests that the reduction of calcification is not the only way by which Pio improves wall elasticity in the VDN model and that calcification is not the only factor responsible for elastic fiber degradation. This is backed up by the observation that the regression analysis of EM/WS versus wall calcium content shows a lower slope for VDN rats.
arterial calcification. However, we have shown in the VDN (ie, an increase in both mean and pulse pressures) amplifies oxidant effects and protection of endothelial function or some effect at the level of the angiotensin II type 1 receptor. Megnien et al showed that hypertension chemical reaction of elastocalcinosis. There are arguments for subsequent reduction in pulsatile wall stress could somehow lowered pulse pressure, and it could be argued that the diastolic pressure in VDN. This would maintain coronary pulse pressure is attributable to attenuation of the fall in accompanied by wall thickening in VDN.

Whatever the mechanism, protection of the elastic fiber protects against calcification and degradation of aortic wall malization of the elastic properties. However, global changes in wall integrity/component and, thus, for the nor-

pronounced enlargement of the aorta) may account for the distribution of tension throughout the wall (eg, less recruitment of stiff collagen fibers because of the less pronounced enlargement of the aorta) may account for the changes in wall integrity/component and, thus, for the normalization of the elastic properties. However, global changes in wall composition such as fibrosis (Pio has no effect on elastocalcinotic arteriosclerosis, thus preventing aortic from degradation maintained aortic wall elastic properties and structural integrity, thus preventing aortic aneurysm. It is interesting to note that dilatation is not accompanied by wall thickening in VDN.

Finally, it should be noted that the beneficial Pio effect on pulse pressure is attributable to attenuation of the fall in diastolic pressure in VDN. This would maintain coronary perfusion and may be relevant in the elderly. Pio significantly lowered pulse pressure, and it could be argued that the subsequent reduction in pulsatile wall stress could somehow attenuate the pressure-induced amplification of the physicochemical reaction of elastocalcinosis. There are arguments for and against this. Megnien et al showed that hypertension (ie, an increase in both mean and pulse pressures) amplifies arterial calcification. However, we have shown in the VDN model that long-term treatment with the calcium entry blocker, isradipine, lowers mesenteric artery calcification but has no effect on blood pressure (or on aortic calcification). This has been discussed previously and the question is still unanswered as to whether increased pressure (and, thus, increased wall stress) amplifies wall elastocalcinosis.

**Perspectives**

In conclusion, we have shown that long-term Pio treatment protects against calcification and degradation of aortic wall elastic fibers via, at least partially, an antiinflammatory mechanism. This improves aortic wall elasticity in a rat model of elastocalcinotic arteriosclerosis, thus preventing elevation of pulse pressure and left ventricular hypertrophy. Our results may be clinically relevant in elderly patients experiencing aortic wall stiffening.

**Acknowledgments**

This work was funded by grants from the French Education, Research and Technology Ministry; the Regional Development Committee (Metz, France); the Greater Nancy Urban Council (Nancy, France); the Pharmacolor Association (Nancy, France); the “Fondation de la Recherche Médicale” (Lorraine Committee, France); the “Ligue contre le Cancer” (54 Committee, France); the “Fondation de France,” Paris; and the “Association de la Recherche contre le Cancer” (ARC No. 5637, Paris). We thank Patrick Limñana (Pharmacology Laboratory), Aurélie Beltz (EA3446, Nancy, France), and Annie Artuso (EA3127, Montpellier, France) for help with histomorphometry, Western blot analysis, and ED-1 immunostaining.

**References**


Pioglitazone Improves Aortic Wall Elasticity in a Rat Model of Elastocalcinotic Arteriosclerosis

Virginie Gaillard, Daniel Casellas, Carole Seguin-Devaux, Hervé Schohn, Michel Dauça, Jeffrey Atkinson and Isabelle Lartaud

Hypertension. 2005;46:372-379; originally published online June 20, 2005;
doi: 10.1161/01.HYP.0000171472.24422.33

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/46/2/372

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at: http://hyper.ahajournals.org//subscriptions/