THORACIC AORTIC STIFFENING ELICITED BY PROLONGED PROPRANOLOL TREATMENT IN AN ANIMAL MODEL

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ABSTRACT
This study assessed the long-term effect of propranolol administration on the elastic and failure properties of the thoracic aorta. Control rats (n=5) received only water with their food, whereas beta-blockade was produced in another 5 rats by administering 100 mg/kg/day propranolol in their drinking water. The animals were sacrificed after 3 months and the descending thoracic aorta was resected, cut into specimens with circumferential (CIRC) and longitudinal (LONG) orientation, and submitted to evaluation of elastic and failure properties. Our observations indicated a stiffer and stronger thoracic aorta in propranolol-treated rats, mostly in the LONG direction, at physiologic and high stresses. Thoracic aortic stiffening, elicited by prolonged b-blocker treatment, warrants further clinical investigation.

KEY WORDS
Biomechanics; b-blockers; elastic properties; rupture; aortic wall; mathematical model.

1. Introduction
There is currently growing interest in the evaluation of the elastic properties of aortic wall and their association to cardiovascular pathology [1,2]. The aorta serves two major tasks: it first acts as a conduit delivering blood from the heart to peripheral tissues and it secondly transforms the pulsatile blood flow caused by ventricular ejection to a continuous flow in the periphery. The elastic properties of vessel wall are crucial to its normal function. The elastic properties of conduit vessels differ strikingly throughout the arterial tree, with more elastic central arteries and stiffer peripheral arteries. This non-uniformity is caused by the histological microstructure of arterial wall, varying among the different parts of the circulation [1-6]. Importantly, the elastic properties of aortic wall assume pivotal roles in the pathogenesis of normal aging and cardiovascular disease, such as hypertension, atherosclerosis, and abnormalities of aortic wall. Aortic compliance is impaired, i.e. decreased, in all these states, and all existing data recommend that early detection of this dysfunction might offer a tool for early identification of cardiovascular pathology [1,2].

Propranolol and other b-adrenergic blockers (BBs) have been long used in the treatment of hypertension, angina pectoris, and various arrhythmias, while the hemodynamic effects of BBs have been used in the management of aortic dissection [7]. Propranolol has also been shown to inhibit aortic root dilatation in patients with Marfan's syndrome when administered in dosages that lower dp/dt [8]. Long-term administration of BBs may therefore protect against abdominal aortic aneurysm (AAA) expansion by reducing the hemodynamic stresses acted on aortic wall. Numerous studies have confirmed that the low rate of AAA expansion among patients receiving BBs was not related to decreased mean, systolic, or diastolic blood pressure [9-11]. Evidence from other studies implied that specific BBs may interact metabolically with aortic tissue [12-14]. Propranolol in particular suppressed the formation of aortic aneurysm in b-aminoproprionitrile fed turkeys [12]; this effect was not dependent on changes in heart rate, blood pressure, or dp/dt [13]. Supplementary data revealed that it augmented the tensile strength of aortic wall by stimulating lysyl oxidase activity and promoting collagen and elastin cross-linking [14]. In view of the above, the influence of propranolol in turkeys fed b-aminoproprionitrile may rely on the direct interaction with b2-receptors situated in non-cardiac vascular tissues.

This study was set to address the effect of prolonged propranolol treatment on the elastic properties of aortic wall in an animal model, as limited information is presently available in the literature. Such information is vital for understanding the consequences of BB therapy for the entire circulation, because propranolol is a pharmaceutical commonly administered to patients with a wide range of pathologies [15].

2. Materials and Methods

2.1 Animal Model, Propranolol Administration, and Tissue Preparation
Ten male Wistar rats (350-400 g) were used in this study. The rats were housed individually in the animal facility at a constant room temperature (19-21°C), relative humidity (55%), under an artificial 12-hour light:12-hour dark
cycle. The animals had access to standard rat chow and water *ad libitum*. The protocol was approved by the Veterinary Directorate of Athens Prefecture and conducted in accordance with the guiding principles of the American Physiological Society and the Greek Presidential Decree 160/1991, issued after the Directive 86/609/EEC.

The animals were randomly assigned into two groups: b-blockade was induced in rats of the first group (n=5), by administering propranolol hydrochloride, 100 mg/kg/day, in their drinking water. The rats of control group (n=5) were age-matched and housed under identical conditions to those of the experimental group, without receiving the drug.

Animals of both groups were sacrificed 3 months from the initiation of propranolol or vehicle treatment. Each rat was sedated with ketamine (90 mg/kg, ip) and xylazine (5 mg/kg, ip), and euthanized with pentobarbital sodium (iv). The chest cavity was entered through a midline incision and the descending thoracic aorta was harvested with care to avoid injury to the aortic wall. CIRC-directed rings and LONG-directed strips were cut, cleaned of periadventitial loose tissues, and used for mechanical evaluation within 2 h post-euthanasia.

### 2.2 Evaluation of Elastic and Failure Properties

Elastic and failure properties were studied with a tensile tester (Vitrodyne V1000 Universal Tester, Liveco Inc, Burlington, VT, USA), as has been described by our group [3,5,16-19]. In brief, specimens were mounted in the grips of the experimental device and subjected to a continuously elevating tensile load along their long axis at a 100-μm/s extension rate until failure of the tissue. Most specimens exhibited two ruptures (see Fig. 1 for a typical example). First ruptured the inner layers of aortic wall and then the outer; data from the first rupture were only evaluated. To sustain normal hydration of aortic specimens, they were immersed in a Krebs-Ringer bath plus 10 μM papaverine, aerated with 95% O₂-5% CO₂ and regulated at 37°C via a heater coil (1130A, PolyScience, Niles, IL, USA).

After placement in the apparatus, the initial dimensions of the aortic specimens were recorded at the no-load state; their initial width and thickness determined optically under a laser beam micrometer (LS-3100, Keyence Corp, Osaka, Japan) with 1-μm resolution. Due to the non-uniform width and thickness of specimens, four measurements were taken and averaged. The extension of specimens and the tensile load applied during the test were measured with a sampling frequency of 50 Hz. 0.25-g resolution load cells equipped the tensile tester for load recording and a rotary encoder with 10-μm accuracy for extension recording.

### 2.3 Data Analysis

Data analysis involved the assessment of engineering strain, engineering stress, and elastic modulus [3,5,16-19]. Engineering (infinitesimal) strain $\varepsilon$ was calculated by subtracting unity from the stretch ratio $\lambda$:

$$\varepsilon = \lambda - 1, \quad \lambda = l/l_0,$$

the latter being the deformed length of aortic specimens at each tensile load divided by their initial length at no-load. Engineering stress $T$ was calculated as the uniaxial load exerted to the specimens divided by their initial width and thickness:

$$T = F/w_0 t_0.$$  \hspace{1cm} (2)

The passive stiffness of aortic tissue was quantified in terms of the elastic modulus $M$, which was calculated as the first derivative of stress over strain:

$$M = dT/d\varepsilon.$$  \hspace{1cm} (3)

Failure stress $T_f$, i.e. tensile strength, and failure strain $\varepsilon_f$, an index of tissue extensibility, were taken as the stress and strain at failure, whereas peak elastic modulus $M_p$ was taken as the maximum slope of stress-strain curve attained prior to failure.

The experimental stress-strain data up to the yield point, i.e. in the elastic domain prior to damage and failure of the aortic tissue, were fitted by a previously-validated power and exponential model [19] (least-squares fitting via the Levenberg-Marquardt algorithm) using Microcal Origin v.8.0 (OriginLab® Corp, Northampton, MA, USA):

$$T = k\varepsilon^q + a(e^{bc} - 1),$$  \hspace{1cm} (4)

in terms of four curve-fit parameters $k$, $q$, $a$, and $b$. The exponential term of this model is negligible when stress is small, so that the power function models the data, while playing a dominant role at large stresses, and the combined

![Fig. 1. Stress-strain data of a CIRC ring (■) and a LONG strip (○) from the thoracic aorta of a propranolol-treated rat. Theoretical curves (Eq. (4)) are plotted with solid lines, and the model parameters were $k=230.1$ kPa, $q=1.657$, $a=0.560$ kPa, $b=6.001$ for CIRC and $k=222.8$ kPa, $q=1.089$, $a=6.440\times10^{10}$ kPa, $b=26.761$ for LONG data. $T_f$, $\varepsilon_f$, and $M_p$ denote failure stress and strain, and peak elastic modulus. $T_f=1398.4$ kPa, $\varepsilon_f=1.320$, $M_p=3688.4$ kPa for CIRC and $T_f=2547.9$ kPa, $\varepsilon_f=0.920$, $M_p=8578.6$ kPa for LONG data.](image-url)
model adequately predicts the physiologic-stress behavior (see Fig. 1 for an example). Dimensionless parameters $q$ and $b$ may be viewed as indices of vessel stiffening under progressive loading, whereas parameters $k$ and $a$, with stress units, as scaling factors. The yield point, defining the upper limit of the elastic domain, was manually selected as that affording the highest correlation coefficient upon fitting the stress-strain data with Eq. (4).

Parameters were expressed as mean ± SEM. Student’s t-test was used to compare among groups and directions with SPSS v13.0 (SPSS Inc, Chicago, IL, USA). Significance was set at $p<0.05$.

3. Results

Stress-strain data of all specimens tested successfully from the descending thoracic aorta of propranolol-treated and untreated rats (control) are shown in Fig. 2, according to specimen direction, exhibiting noticeable variations. The central body of curves from propranolol-treated rats was displaced upwards than that of control in the LONG, but not CIRC direction, for which curves were superimposed. In both rat groups, curves for the LONG direction were on average displaced leftwards and upwards than those for the CIRC direction.

Failure stress and peak elastic modulus of descending thoracic aortas from propranolol-treated rats did not differ from control in the CIRC direction, but were significantly higher in the LONG direction (Table 1). No statistically significant variation among propranolol-treated and control rats was found in failure strain in either CIRC or LONG specimens. For each animal group, failure stress and peak elastic modulus of LONG specimens were significantly higher than those of the respective CIRC specimens, while the opposite was true for failure strain.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>$T_f$ (kPa)</th>
<th>$\varepsilon_f$ (-)</th>
<th>$M_p$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propranolol-Treated</td>
<td>1267.0</td>
<td>1.311</td>
<td>3039.1</td>
</tr>
<tr>
<td>(n=5)</td>
<td>± ±</td>
<td>± ±</td>
<td>± ±</td>
</tr>
<tr>
<td>CIRC specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1285.5</td>
<td>1.193</td>
<td>3275.9</td>
</tr>
<tr>
<td>(n=5)</td>
<td>± ±</td>
<td>± ±</td>
<td>± ±</td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.2</td>
<td>0.2</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Propranolol-Treated</td>
<td>2497.7</td>
<td>0.919</td>
<td>9327.4</td>
</tr>
<tr>
<td>(n=5)</td>
<td>± ±</td>
<td>± ±</td>
<td>± ±</td>
</tr>
<tr>
<td>LONG specimens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1928.6</td>
<td>0.944</td>
<td>6500.3</td>
</tr>
<tr>
<td>(n=5)</td>
<td>± ±</td>
<td>± ±</td>
<td>± ±</td>
</tr>
<tr>
<td>$p$</td>
<td>&gt;0.2</td>
<td>&gt;0.02</td>
<td>0.02</td>
</tr>
</tbody>
</table>

$T_f$ = failure stress; $\varepsilon_f$ = failure strain; $M_p$ = peak elastic modulus; $p = p$-value for statistical comparison between groups (unpaired Student’s t-test). * = $p<0.05$ and ** = $p<0.01$ (paired Student’s t-test), directional variations. Parameters are given as mean ± SEM.

The characteristic nonlinear shape of our stress-strain data was evident in both directions and for specimens from propranolol-treated and untreated rats. The corresponding regression curves, derived by fitting the data to Eq. (4), afforded very accurate fits with correlation coefficients over 0.99. Model parameters of the stress-strain data up to yield point are summarized in Table 2. Minor propranolol-effected differences were noted in parameter $q$ for CIRC specimens, and parameter $b$ for LONG specimens, both being higher in propranolol-treated than control rats, while parameters $k$ and $a$ were unchanged. Differences in $q$, $a$, and $b$ among directions were significant. These data imply that LONG specimens were significantly stiffer than CIRC ones at physiologic and high stress levels. Importantly, the thoracic aorta of propranolol-treated rats was stiffer than...
that of untreated rats at physiologic stresses in the CIRC, and at high stresses in the LONG direction.

4. Discussion

It is well known that BBs counteract the effects that catecholamines have at the b-adrenergic receptor, lessening their interactions, suspending the production of cyclic AMP, and eventually restraining calcium influx across the sarcolemma and calcium release by the sarcoplasmic reticulum [15]. This competition elicits reduced myocardial contractility (negative inotropic effect) and heart rate as a result of the decreased automaticity in sinus node (negative chronotropic effect) and the slowing of conduction in the atrioventricular node (negative dromotropic effect) [15].

Abundant studies have demonstrated that therapy with BBs reduces the rate of aortic growth [20-23] and clinicians regard them as the standard of care. The presumed mechanisms underlying their action, i.e. reduced proximal aortic shear stress and heart rate, are plausible according to the pathophysiologic analysis; nonetheless, treatment with BBs does not prevent the attainment of important clinical end-points, such as aortic regurgitation, surgery, dissection, and death.

Little information is currently available with regard to the effects of BBs on the elastic properties of arterial wall. Earlier studies have considered the acute outcome of b-adrenergic blockade on aortic elasticity but with conflicting results. Blood pressure reduction in healthy subjects was documented in one study [24] after BB therapy with unaltered aortic distensibility and pulse wave velocity, whereas in another [25], distensibility and stiffness in turn rose and fell, owing to the lowering of pressure. Our group has lately examined the time-course of propranolol-effected changes in the elastic properties of aortic wall [16], and disclosed that thoracic aorta became gradually stiffer with the duration of treatment at physiologic stress levels, with differences attaining significance at 3 months. A limitation of this study was that the elastic properties were determined in the LONG direction of the vessel, whereas those in the CIRC direction are physiologic relevant, determining the arterial compliance and pulse pressure.

Characterization of the mechanical properties of large arteries, particularly of the aorta, is complex and presents many challenges, both on theoretical and technical aspects. Indeed, arteries exhibit marked anisotropy, non-linearity, and powerful adaptive mechanisms [1,2]. Further, no single arterial segment has identical mechanical properties and it is impossible to extrapolate segmental arterial properties to the whole arterial tree. In the present study, we addressed anisotropy and found distinctly dissimilar elastic and failure properties in the two principal directions; we also found the influence of chronic propranolol treatment to differ in the two axes. The nonlinearity of stress-strain data was taken into account, utilizing a previously-validated model [19], that uses a power function to capture the low-stress elastic response and combines it with an exponential function to capture the high-stress response. The evaluation of model parameters representing our data demonstrated increased stiffness of the thoracic aorta in propranolol-treated rats at medium and high levels of stress.

Table 2
The effect of 3-month propranolol administration on the model parameters of thoracic aorta

<table>
<thead>
<tr>
<th></th>
<th>CIRC specimens</th>
<th>LONG specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>k (kPa) q (-) a (kPa) b (-)</td>
<td>k (kPa) q (-) a (kPa) b (-)</td>
</tr>
<tr>
<td>Propranolol-</td>
<td>185.0 1.409 0.247 7.609</td>
<td>154.9 0.972 0.002 21.432</td>
</tr>
<tr>
<td>Treated (n=5)</td>
<td>± ± ± ±</td>
<td>± ± ± ±</td>
</tr>
<tr>
<td>Control (n=5)</td>
<td>166.8 1.159 1.829 7.134</td>
<td>119.7 0.872 0.026 15.979</td>
</tr>
<tr>
<td>p</td>
<td>&gt;0.2 &gt;0.2 &gt;0.2 &gt;0.2</td>
<td>&gt;0.2 &gt;0.2 &gt;0.2 &gt;0.2</td>
</tr>
</tbody>
</table>

k, q, a, and b are model parameters; p = p-value for statistical comparison between groups. * = p<0.05 and ** = p<0.01, directional-dependent differences. Parameters are given as mean ± SEM.
amplifies central pulse pressure and the load on the left ventricle, reduces ejection fraction, and raises myocardial oxygen demand [1,2,26]. It associates with left ventricular hypertrophy in normotensive and hypertensive patients [27,28], a known risk factor for congestive heart failure and cardiovascular events [29]. Furthermore, increased aortic stiffness aggravates cardiovascular morbidity and mortality owing to an elevation of systolic blood pressure that raises left ventricular afterload, and a decrease in diastolic blood pressure that alters coronary perfusion [1,2,26]. It is also correlated with atherosclerosis [30,31], probably through the effects of cyclic stress on arterial wall thickening [32,33]. At last, increased aortic stiffness is an independent predictor of all cause and cardiovascular mortality in patients with essential hypertension [26] and with end-stage renal disease [35]. When accompanying cardiovascular risk factors and age, the histological manifestations of stiffening include elastin fiber fragmentation, collagen accumulation, fibrosis, inflammation, medial smooth muscle cell necrosis, calcifications, and diffusion of macromolecules within the arterial wall [35-37]. Future investigations are warranted to ascertain the histological modifications taking place in response to propranolol administration.

5. Conclusion

It is inferred that chronic pharmacological blockade of b-adrenergic receptors with propranolol induced significant variations in the elastic properties of thoracic aorta in an experimental model. The aortic wall suffered stiffening at physiologic and high levels of stress. Alterations in aortic distensibility, in response to chronic administration of BBs, should be taken into consideration in clinical practice.

Acknowledgement

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References


