Novel Arterial Pathology in Mice and Humans Hemizygous for Elastin

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Abstract

Obstructive vascular disease is an important health problem in the industrialized world. Through a series of molecular genetic studies, we demonstrated that loss-of-function mutations in one elastin allele cause an inherited obstructive arterial disease, supravalvular aortic stenosis (SVAS). To define the mechanism of elastin’s effect, we generated mice hemizygous for the elastin gene (ELN +/−). Although ELN mRNA and protein were reduced by 50% in ELN +/− mice, arterial compliance at physiologic pressures was nearly normal. This discrepancy was explained by a paradoxical increase of 35% in the number of elastic lamellae and smooth muscle in ELN +/− arteries. Examination of humans with ELN hemizygosity revealed a 2.5-fold increase in elastic lamellae and smooth muscle. Thus, ELN hemizygosity in mice and humans induces a compensatory increase in the number of rings of elastic lamellae and smooth muscle during arterial development. Humans are excessively sensitive to reduced ELN expression, developing profound arterial thickening and markedly increased risk of obstructive vascular disease. (J. Clin. Invest. 1998. 102:1783–1787.) Key words: elastin • cardiovascular models • mice, transgenic • vascular disease • compliance

Introduction

Obstructive vascular disease is the predominant cause of mortality and morbidity in developed nations (1). Extensive research has implicated key pathogenic roles for lipids and growth factors, but it is likely that other factors participate in the pathogenesis of these disorders. In a series of molecular genetic experiments, we demonstrated that a human obstructive vascular disorder, supravalvular aortic stenosis (SVAS), is associated with hemizygosity of the elastin gene (ELN) (2–6). Mutations in genes encoding other structural proteins, including collagen and fibrillin, have also been associated with vascular disease, but these genetic abnormalities cause dissection and degeneration, not obstruction (7, 8). The pathogenic mechanisms underlying ELN mutations are not understood.

Elastin is the dominant arterial extracellular matrix protein, comprising 50% of the dry weight of the aorta (9). Encoded by a single gene on human chromosome 7q11.23, elastin expression is largely confined to the third trimester of fetal development and early postnatal years. Elastin is synthesized by smooth muscle, secreted as a soluble monomer, tropoelastin, and organized into insoluble polymers that form concentric rings of elastic lamellae around the arterial lumen. Each elastic lamella alternates with a ring of smooth muscle, forming a lamellar unit. Elastic lamella provides the resilience that arteries need to absorb hemodynamic stress of cardiac systole and to release this energy in the form of sustained blood pressure during diastole. The number of lamellar units is thought to be species specific, fixed, and genetically predetermined (10, 11).

Previously, human pathologic studies of SVAS described medial necrosis, fibrosis, and disorganization, a common end-stage pathology of many vascular diseases (12, 13). Identifying the mechanism of disease through these studies was hampered by the inability to separate cause and effect. To define the pathogenic mechanism underlying SVAS, we characterized mice hemizygous for ELN. This work demonstrates that the number of lamellar units is neither fixed nor species specific, but is modulated by the level of ELN expression during development. We show that humans are extremely sensitive to reduced ELN expression, developing profound thickening of the arterial wall.

Methods

Northern analysis. Poly(A)+ RNA was extracted from the visceral organs of the thorax from mice at birth using a Micro-Fast Tact Kit (Invitrogen, Carlsbad, CA). RNA was electrophoresed on a 1.0% denaturing agarose gel, transferred to Hybond filter (Amersham, Arlington Heights, IL), and hybridized with a 32P-labeled 0.85-kb fragment of mouse ELN cDNA. Filters were rehybridized with a 1.5-kb fragment of human cardiac actin cDNA. Intensity of bands was measured by PhosphorImager analysis. ELN expression in ELN +/+ and ELN +/− mice was standardized to cardiac actin expression and compared.

Electron microscopy. Ascending thoracic aortae were dissected from mouse pups at birth after cardiac perfusion with 3% glutaraldehyde. Aortic segments were sequentially stained with osmium tetroxide, tannic acid, and uranyl acetate, then dehydrated and embedded in Epon. Thin sections (60-nm) were counterstained with uranyl acetate and lead citrate and examined on a Jeol 1200 electron microscope (14).

Histological examination. Mice were fixed overnight in either 4% paraformaldehyde or methyl Carnoys at 4°C and embedded in paraffin. Sections were stained with hematoxylin and eosin and Hart stain for elastin. Individuals blinded to the genotype counted lamellar units.
elastic lamellae. Note that elastic lamellae are abnormal and oriented smooth muscle cells are interposed between well developed thinner in cross-sections from arteries. Bar, 3.0 μm.

Results

To define the role of ELN hemizygosity in arterial disease, we generated mice hemizygous (ELN +/-) for an ELN null mutation using homologous recombination in embryonic stem cells (17). Three independent recombinant cell lines containing a 4.0-kb deletion of the promoter and exon 1 of ELN were used to generate chimeras. Chimeras were mated to generate ELN +/- mice. ELN +/- mice were identical to ELN +/+ mice in gross appearance, behavior, and life expectancy. Northern analysis of ELN +/- mice revealed a 47% decrease in ELN mRNA when compared with ELN +/+ mice at birth (Fig. 1, a and b). To determine if the structure of elastic lamellae in ELN +/- mice was affected by reduced ELN expression, we examined aortic cross-sections by electron microscopy. Elastic lamellae in ELN +/- aortae (Fig. 1, c and d) were ~ 50% thinner than those in ELN +/- aortae (Fig. 1, e and f). These data indicate that elastic lamellae in ELN +/- mice are structurally abnormal.

To determine the physiologic consequences of structural changes observed in ELN +/- mice, we measured aortic diameter and extensibility at varying intraluminal pressures. Despite differences in ELN mRNA expression and protein deposition, at a physiologic pressure of 100 mmHg ELN +/+ and ELN +/- aortae had similar extensibilities (23.6 ± 6.0% vs. 22.5 ± 0.7%) (Fig. 2) (18). At 125 mmHg and above, however, the pressure–diameter curves diverged with a marked reduction in extensibility of ELN +/- aortae (at 125 mmHg, 18.8 ± 1.5% for ELN +/- aortae vs. 11.2 ± 4.4% for ELN +/- aortae, P < 0.05). The extensibility of ELN +/- pulmonary arteries was similar to controls within a broad pressure range (0–50 mmHg) surrounding normal physiologic pressure (mean pressure = 10 mmHg). These data indicate that ELN +/- mice maintain extensibility at physiologic pressures.

To understand the structural determinants enabling ELN +/- aortae to maintain normal extensibility at physiologic pressures, we examined aortic cross-sections by electron microscopy. Elastic lamellae in ELN +/- aortae are significantly less than ELN +/+ aortae. (b) Extensibility at varying pressures. The extensibilities of ELN +/- (black bars) and ELN +/- aortae (white bars) are similar at physiologic pressure (100 mmHg). However, at elevated pressures (125 and 150 mmHg) extensibility of ELN +/- aortae is significantly decreased compared with controls. *Statistically significant differences, P < 0.05.

Figure 1. (a and b) Northern analysis of mRNA from ELN +/+ and ELN +/- mice at birth for ELN (a) and cardiac actin (b) expression. There is a 47% decrease in ELN mRNA in ELN +/- mice by PhosphorImager analysis. (c–f) Electron micrographs of ascending aortic cross-sections from ELN +/+ (c and e) and ELN +/- (d and f) mice at birth. Subendothelial (c and d) and medial sections (e and f) for both genotypes are compared. In ELN +/- aortae, circumferentially oriented smooth muscle cells are interposed between well developed elastic lamellae. Note that elastic lamellae are abnormal and ~ 50% thinner in ELN +/- aortae. Bar, 3.0 μm.

Figure 2. Extensibility of ELN +/+ and ELN +/- aortae. (a) Pressure–diameter curve. At pressures 100 mmHg and below, ELN +/- aortae (filled circles) have diameters similar to ELN +/- aortae (open squares). At pressures above 100 mmHg, the diameters of ELN +/- aortae are significantly less than ELN +/- aortae. (b) Extensibility at varying pressures. The extensibilities of ELN +/- (black bars) and ELN +/- aortae (white bars) are similar at physiologic pressure (100 mmHg). However, at elevated pressures (125 and 150 mmHg) extensibility of ELN +/- aortae is significantly decreased compared with controls. *Statistically significant differences, P < 0.05.

Vascular extensibility. Arteries studied were cannulated and mounted on the pressure myograph (15). The vessel was transilluminated under an inverted microscope connected to a CCD camera, allowing the continuous recording of the outer diameter of the vessel. In the ascending aorta, intravascular pressure was increased from 75 to 175 mmHg by steps of 25 mmHg (0–50 mmHg by steps of 10 mmHg for the left pulmonary artery), and the arterial diameter was recorded. Extensibility was calculated using the following formula with 125 mmHg as an example: extensibility = [(diameter at 150 mmHg – diameter at 100 mmHg)/(diameter at 100 mmHg)] × 100 (16). Diameter statistical analysis was assessed by a four-way ANOVA followed by least significance difference test for post-ANOVA paired comparisons. Extensibility statistical analysis was assessed by the nonparametric Mann-Whitney U test. P values below 0.05 were considered statistically significant.

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pressure despite a 50% decrease in elastin, we examined the arterial structure. We discovered that aortae dissected from 
ELN +/– mice (5–14 mo) had additional lamellar units. Consist-
ent with previous work, we found that ELN +/+ aortae had 5.4±0.5 (descending) and 8.4±0.5 (ascending) layers of elas-
tic lamellae (Table I and Fig. 3) (10, 19). By contrast, cross-
sections of ELN +/– mice revealed an increase in the num-
ber of elastic lamellae to 7.3±0.6 (descending) and 10.5±0.5 (as-
cending) layers, respectively (P < 0.005). This represented an in-
crease of 35% and 25% for the descending and ascending 
aortae of ELN +/– mice, respectively. Similar changes were 
observed in the pulmonary artery with an average of 7.5±0.7 
lamellar units in ELN +/– mice vs. 6.1±0.2 in the ELN +/+ 
mice (P < 0.005). These changes were also apparent at birth. 
Our data demonstrate that ELN +/– mice develop additional 
rings of elastic lamellae and smooth muscle. Thus, the number 
of lamellar units in an arterial wall is not fixed or species spe-
cific.

To determine if developmental changes observed in the 
aortae of ELN +/– mice were also present in humans, we ex-
amined aortic segments from individuals with SVAS (n = 2 for 
fected, n = 3 for controls; Fig. 4 and data not shown). Previ-
sous studies focused only on arterial sections affected by dis-
crete stenosis and showed subendothelial accumulation of 
cells, hypertrophy of smooth muscle, disruption of elastic fi-
bers, and fibrosis (12, 13). We studied regions of the aorta that were free of discrete stenosis. The number of lamellar units in 
control was consistent with previous reports (10, 20). By con-
trast, the aortic wall of individuals with SVAS was thicker and 
contained 2.5-fold more lamellar units (152±27.6 vs. 62±8.7; P < 
0.025). These data indicate that humans, like mice, respond to 
reduced elastin content during development by increasing the 
number of lamellar units.

### Discussion
It was thought previously that the number of lamellar units in a 
developing arterial wall is fixed and species specific (9, 10). 
Our work demonstrates that this concept is incorrect. We 
found that ELN +/– mice develop arteries with a 25–35% in-
crease in the number of lamellar units. Examination of arterial 
specimens obtained from individuals with SVAS, a human dis-
order caused by ELN hemizygosity, revealed a 2.5-fold in-
crease in the number of elastic lamellae. Thus, the number of 
lamellar units in an arterial wall is not fixed or species spe-
cific and is modulated by ELN expression.

Elastin’s effect on arterial lamellar development likely in-
volves smooth muscle cells sensing increased wall stress. The 
reduced ELN mRNA and thinning of each elastic lamella ob-
served in ELN +/– mice would cause reduced extensibility in 
each lamella. These changes would lead to increased arterial 
wall stress, which is determined by arterial pressure and diam-
eter and inversely proportional to the number of lamellar units 
and the tensile strength of each unit (21). However, the exten-
sibility and diameter of ELN +/– arteries were normal at 
physiologic pressures. Thus, mice with abnormal elastic fibers 
maintain arterial extensibility by increasing the number of 

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**Table I. Number of Lamellar Units in ELN +/+ and ELN +/– Aortae**

<table>
<thead>
<tr>
<th>Mouse ID</th>
<th>Genotype</th>
<th>Age</th>
<th>Ascending aorta</th>
<th>Descending aorta</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL6</td>
<td>ELN +/+</td>
<td>5 mo</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>390-3</td>
<td>ELN +/+</td>
<td>12 mo</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>390-10</td>
<td>ELN +/+</td>
<td>14 mo</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>390-11</td>
<td>ELN +/+</td>
<td>14 mo</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>1002-7</td>
<td>ELN +/+</td>
<td>4 mo</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>1029-8</td>
<td>ELN +/+</td>
<td>5 mo</td>
<td>8</td>
<td>5</td>
</tr>
<tr>
<td>1045-3</td>
<td>ELN +/+</td>
<td>5 mo</td>
<td>9</td>
<td>5</td>
</tr>
</tbody>
</table>

Average 8.4±0.5 5.4±0.5

390      | ELN +/– | 12 mo| 11           | 7               |
| 390-8    | ELN +/– | 14 mo| 11           | 7               |
| 390-9    | ELN +/– | 14 mo| 11           | 7               |
| 705-7    | ELN +/– | 7 mo | 11           | 8               |
| 720-2    | ELN +/– | 7 mo | 10           | 8               |
| 720-6    | ELN +/– | 7 mo | 10           | 6               |
| 1029-4   | ELN +/– | 6 mo | 11           | 8               |
| 1029-5   | ELN +/– | 6 mo | 10           | 7               |
| 1029-6   | ELN +/– | 5 mo | 10           | 7               |
| 1029-7   | ELN +/– | 5 mo | 10           | 7               |
| 1045-1   | ELN +/– | 6 mo | 11           | 7               |
| 1045-4   | ELN +/– | 6 mo | 10           | 8               |

Average 10.5±0.5 7.3±0.6

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**Figure 3.** Hart stains of the ascending (a and b) and descending (c–f) aortae in ELN +/+ (a, c, and e) and ELN +/– (b, d, and f) mice.

Cross-sections are at the level where the pulmonary artery courses 
behind the ascending aorta. In this example there are 8 and 6 elastic lamellae in the ascending and descending aortae of the ELN +/+ 
mouse, respectively, compared with 11 and 8 in the ELN +/– mouse, 
respectively, indicating an inverse relationship between ELN expres-
sion and the number of elastic lamellae. Lower magnification of the 
descending aorta (e and f) demonstrates that the inner and outer di-
ameters of ELN +/+ and ELN +/– aortae are similar.
lamellar units during development. Our model is further supported by anatomic and physiologic studies showing that the relationship between wall stress and the number of lamellar units in an artery is remarkably constant across species despite enormous variation in arterial diameter and stress (10). In addition, we observed no increase in the number of lamellar units when ELN+/− aortae were cultured in the absence of hemodynamic stress (17 and data not shown). Thus, the physiologic force of wall stress is a key determinant of arterial development.

Our work defines a novel pathology in a human obstructive arterial disease, SVAS. Because of arterial pathology in ELN+/− mice, we examined aortic sections from individuals with SVAS and found a compensatory increase in arterial lamellar units. As human arteries are larger and wall stress is greater (110,000 dynes/cm in humans vs. 7,800 dynes/cm in mice) the increase in humans is profound (2.5-fold increase in humans). The increase in the smooth muscle cells needed to form additional layers likely outstrips blood supply, leading to medial necrosis and fibrosis. These changes in the medial wall would stimulate recurrent injury and repair resulting in the focal stenosis observed in SVAS (12, 13).

Our data indicate that the number of lamellar units can only be modulated by wall stress early in development. During gestation when pulmonary vascular pressures are similar to systemic pressures, pulmonary arteries respond to ELN hemizygosity by increasing the number of lamellar units. Despite a sharp decrease in hemodynamic stress with onset of respiration at birth, the number of lamellar units in the pulmonary artery does not change. Later in life, lamellar structure is fixed and medial hypertrophy is the primary arterial response to increased hemodynamic stress (22).