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Alteration of Elastic Architecture in the Lathyritic Rat Aorta Implies the Pathogenesis of Aortic Dissecting Aneurysm

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Weanling Sprague-Dawley rats received β-aminopropionitrile (BAPN) and/or hypertensive treatment, namely, heminephrectomy and administration of deoxycorticosterone acetate-NaCl. The BAPN-treated rats (lathyritic rats) died of dissecting aneurysm, and the victims with hypertensive treatment was greater in number and died earlier than those without the treatment, indicating that the rise of blood pressure promoted the onset of dissecting aneurysm. The elastic architecture of the ascending aortic media was examined not only by transmission electron microscopy with tannic acid stain and/or toluidine blue O stain, but also by scanning electron microscopy after hot formic acid treatment, and the area of interlaminar elastic fibers were morphometrically analyzed by a point counting method using transmission electron microscopic photographs. In the lathyritic rats, interlaminar elastic fibers showed a significant reduction compared with the control rats, and elastic fibers tended to become round-shaped and were frequently spotted with glycosaminoglycan, which suggest a disturbance of elastogenesis. On the other hand, elastic laminae were not disrupted and smooth muscle cells were well preserved. These results suggest that the alteration of the elastic architecture causes an unstable connection between each elastic lamina, and is related to the initiation and the progression of dissecting aneurysm. (Am J Pathol 1992, 140:959–969)

The pathogenesis of dissecting aneurysm is still not well understood. Recent histopathologic studies have demonstrated that cystic medial necrosis, which had been believed to be a most important pathognomonic change, was not so frequently found in the autopsied cases of dissecting aneurysm.[1–3] Investigating the elastic architecture of the ascending aorta in type A dissecting aneurysm (Stanford classification,[4] namely, a dissection from ascending to descending or abdominal aorta) by scanning electron microscopy, we reported a reduction of the interlaminar elastic fibers in the outer media, whereas the elastic laminae were preserved.[5] We hypothesized that the alterations of elastic architecture in the aortic media may play an important role in the initiation and progression of the dissecting aneurysm. Then, in the present study, we investigated the structure of the aortic elastin in the β-aminopropionitrile fumarate (BAPN) treated rat, which is the most successful model of dissecting aneurysm,[6] with transmission and scanning electron microscopies.

In human cases of dissecting aneurysm, hypertension is frequently associated,[3] but it is not well understood how hypertension is actually related to the disease. In the present study we attempted to evaluate the role of hypertension in the pathogenesis of the disease.

Materials and Methods

Grouping and Treatments of Rats

Three-week-old male Sprague-Dawley rats (Jcl:SD, CLEA Japan INC., Tokyo, Japan) weighing 50–60 g were divided into four groups with 10 rats in each group as follows.

Group A

This group consisted of control rats that were subjected to a sham operation under ether anesthesia at 3

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weeks of age. They were given commercial feed pellets (CLEA Japan, Inc.) and autoclaved water for drinking throughout the study. After 4 days of the operation, they received a daily subcutaneous injection of cottonseed oil (Sigma, St. Louis, MO) 5 days per week.

Group B

This group consisted of the hypertensive rats that were subjected to left nephrectomy under ether anesthesia at 3 weeks of age. They were given commercial feed pellets throughout the study and autoclaved water for drinking until 3 days after the operation. After 4 days of the operation, drinking water was replaced by 1% NaCl, and the rats received a daily subcutaneous injection of 15 mg/kg of deoxycorticosterone acetate (DOCA, Sigma, St. Louis, MO) suspended in cottonseed oil (Sigma, St. Louis, MO) 5 days per week.

Group C

This group consisted of lathyritic rats without hypertensive treatment. They were treated the same as group A, except for being fed with 0.4% BAPN (Sigma, St. Louis, MO) mixed into commercial feed pellets after four days of the operation. The feed pellets were prepared by CLEA Japan Inc. (Tokyo, Japan),

Group D

This group consisted of lathyritic rats with hypertensive treatment. They were treated the same as Group B, except for being fed with 0.4% BAPN mixed into commercial feed pellets after 4 days of the operation.

Each rat was housed in individual cages and the amount of ingested food was measured every day. Body weight was measured twice a week. Systolic blood pressure was measured under unanesthetic conditions by the indirect tail cuff method (USR-105-R, Ueda Electric Works LTD., Tokyo, Japan) once or twice a week. The rats that did not die of dissecting aneurysm or debilitation during an experimental course were killed at 9 weeks of age with intraperitoneal injection of an overdose of sodium pentobarbital.

Morphologic Examinations

In the rats that died of dissecting aneurysm, a rupture of the wall prevented perfusion-fixation of the aorta. Therefore, all the specimens taken from Groups A to D were immersion-fixed as follows. Three serial strips, which were about 0.2 cm width and apart from the dissecting part, were taken from the ascending aorta of 0.4 cm and were more distal to the aortic valve ring and fixed with 20% formalin or 3% glutaraldehyde. One of these strips was used in a light microscopic examination and sections parallel and vertical to long axis of aorta were stained with H&E, Weigert's elastic fiber, elastica van Gieson, Azan and alcian-blue stains.

The other two strips were used for scanning and transmission electron microscopic examinations as described later. A three-dimensional elastic architecture was observed by scanning electron microscopy after hot formic acid treatment, according to Wasano and Yamamoto.[7] Briefly, the specimens were immersed in about 15 ml of 88% of formic acid in the plastic bottle at 45°C. After 7 days, the remaining elastin was carefully washed several times with 0.002 N HCl. The washing solution was removed as far as possible and the bottle was soaked in liquid nitrogen. The frozen and fractured elastin was freeze-dried by a freeze-dryer (Multi-Dry, FTS Systems, Inc., Stone Ridge, NY). After the dried specimen was affixed on a copper plate (Oken Shoji Co. Tokyo, Japan) with double-sided sticky tape, it was coated with gold-palladium alloy by a coating apparatus (JFC-1500, JEOL, Tokyo, Japan) and examined by a scanning electron microscope (JSM-35CF, JEOL, Tokyo, Japan).

The elastin of aortic media was also observed by transmission electron microscopy with tannic acid stain. Specimens obtained from the anterior part of the ascending aorta were postfixed with 2% osmium tetroxide. After dehydration with ethanol and propylene oxide, they were carefully oriented so as to obtain sections that were parallel to long axis of aorta and crossradial to the vessel wall, and embedded in Epon 812. According to Kajikawa et al,[8] ultrathin sections were stained with tannic acid-uranyl acetate solution (1% tannic acid, 1.7% paranitrophenol and 0.08% uranyl acetate in distilled water), and lead citrate, and observed with a transmission electron microscope (JEM 1200-EX, JEOL, Tokyo, Japan).

In the rats that died of dissecting aneurysm, the dissected part of the aorta was also observed by light microscopy and scanning electron microscopy in the same manner as described earlier.

The right kidney and the heart are examined light microscopically with H&E, elastica van Gieson, and Azan stains.

Morphometric Analysis of Interlaminar Elastin

Alterations of interlaminar elastic fibers in Groups A to D were morphometrically analyzed using transmission electron microscopic photographs. Inner second or third layer and outer second or third layer of the media were photographed at the same magnification (× 5,000), and

the area of interlaminar elastic fibers was assessed by the point counting method[9] as indicated in Figure 1. Three hundred and seventy one points of 1 mm diameter were marked on an area of 2.8 by 9.8 cm on a clear cellurose sheet. The points were regularly arranged at the corners of equilateral triangles with a space of 2 mm. The sheet was placed on a photograph and then a count was made of the number of points overlying the interlaminar elastin. Three fields of the inner media and three fields of the outer media were analyzed in each rat, and their averages were regarded as the values of the area of the interlaminar fibers in the inner and outer media of the rat, respectively.

Morphometric Analysis in Perfusion-fixed Aorta

Ten 3-week-old rats were treated the same as Group A and nine 3-week-old rats were treated the same as Group C. At 7 weeks of age, after the rats were anesthetized with intraperitoneal injection of sodium pentobarbital (40 mg/kg), the aortas were briefly washed with 0.01 M phosphate-buffered saline, pH 7.2, and then perfusion-fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, at 100 mm Hg from the left ventricle. The anterior part of the ascending aorta was processed for transmission electron microscopy and the area of interlaminar elastin was morphometrically analyzed in the same manner as described earlier, except for the use of a cellurose sheet that had the same numbers of points (371 points) marked on a long and narrow area (1.9 × 14.7 cm).

Two other specimens were taken from the ascending aorta and examined by light and scanning electron microscopies in the same manner as described earlier.

Examination of Elastic Architecture in Low Body Weight Rat

To compare the elastic architecture in low body-weight rats to that of control rats that were fed *ad libitum* and ate 20.0 g/day on the average, ten 3-week-old rats were fed with a restricted amount of commercial feed pellets (3–6 g/day). They were killed at 9 weeks of age with intraperitoneal injection of overdose of sodium pentobarbital and the aortas were immersion-fixed with 3% glutaraldehyde. The ascending aorta was observed by light and transmission electron microscopies in the same manner as described earlier. The area of interlaminar elastin was morphometrically analyzed and compared with that of Group A.

Observation of Correlation between Elastin and Glycosaminoglycan

Three 3-week-old rats were treated the same as Group A and three 3-week-old rats were treated the same as Group C, and then they were killed at 9 weeks of age with an intraperitoneal injection of an overdose of sodium pentobarbital. According to Fornieri et al,[10] GAG was stained with toluidine blue 0, and the distribution of GAG and the correlation between elastin and GAG were observed with a transmission electron microscope. Briefly, specimens taken from the ascending aorta were fixed with 3% glutaraldehyde in Tyrode's solution, pH 7.2, containing 0.1% toluidine blue 0 (Merck, Darmstadt, Germany) for 24 hours at room temperature. They were then washed in the same buffer containing 0.05% toluidine blue 0 for 1 hour and postfixed with 1% osmium tetroxide in Tyrode's solution, containing 0.05% toluidine blue 0 for 2 hours at room temperature. After dehydration with ethanol and

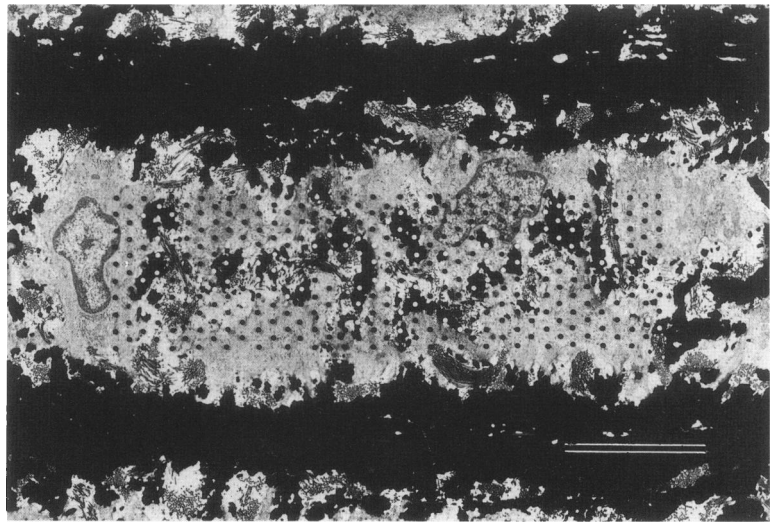

Figure 1. *Methods of measuring the area of interlaminar elastic fibers. The area of interlaminar elastic fibers were measured by a point counting method as described in the Materials and Methods. The points overlying interlaminar elastin were represented in white and the other in black in this figure. Bar represents 5 μm.*

propylene oxide, they were embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and in some sections, a tannic acid stain was superimposed.

Statistical Analysis

The data were represented as the means ± standard deviations. A one-way analysis of variance was used to determine the statistical significance in the area of interlaminar elastin among the Groups A to D. When significance was detected, a comparison between the two groups was performed using modified *t*-statistics.[11] A paired *t*-test was used in the comparison of the area of interlaminar elastin between inner and outer media of each group. In the other comparisons of two statistical values, Student's unpaired *t*-test was used to determine the statistical significance.

Results

General Condition and Body Weight

The general condition of Group A was good and the body weights gradually increased to reach about 310 g by the end of the experiment (Figure 2). In Group B, the rats became pale and body weights decreased when systolic blood pressure became 170 mm Hg or more. Their general condition gradually became worse and one rat died of debilitation at 8 weeks of age. The hearts and the right kidneys of the rats in Group B were generally hypertrophic, and light microscopic examinations revealed prominent fibromuscular thickening of muscular arteries and arterioles of the kidney. In five of them, including the rat which died of debilitation, the arterioles of the kidney also showed fibrinoid degeneration, suggesting that these rats were affected by malignant hypertension. In Groups C and D, the general conditions were bad and skeletal abnormalities, such as scoliosis, appeared. The body weights increased slowly and never exceeded 150 g. One rat in Group C and one rat in Group D died of debilitation at 7 and 8 weeks of age, respectively. Autopsy examinations of these two rats did not reveal any causative morphologic changes, such as dissecting aneurysm and infections. In Group D, the hearts were not hypertrophic. Although the right kidneys showed a compensatory hypertrophy, light microscopic examinations revealed neither fibromuscular thickening nor a fibrinoid degeneration of the arterioles.

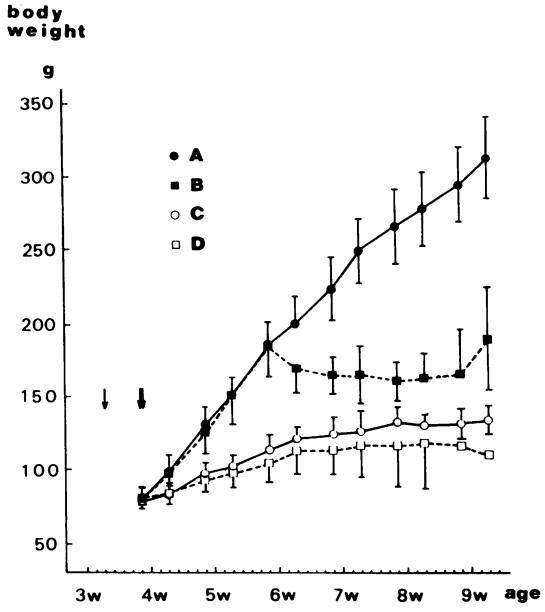

Figure 2. *Time sequential change of the body weight of Sprague–Dawley rats.* **A**: *Control rats.* **B**: *Hypertensive rats.* **C**: *Lathyritic rats without hypertensive treatment.* **D**: *Lathyritic rat with hypertensive treatment. The single and double arrows indicate the time of heminephrectomy and the start of BAPN feeding and administration of DOCA and NaCl, respectively, (Mean ± SD).*

Onset of Dissecting Aneurysm

Four rats in Group C and eight rats in Group D died of a rupture of the dissecting aneurysm (Table 1, Figure 3). The dissection mainly occurred in the aortic arch either with or without involving the distal part of the ascending aorta and/or proximal part of the descending aorta. There was no difference in the location of dissection between Groups C and D. An intimal tear was recognized in all the 12 cases. The victims in Group D died earlier than those of Group C. The mean of the period from the start of BAPN feeding to death in Group D was about 21 days, and it was significantly shorter than that in Group C, which was about 30 days (Table 1). The mean BAPN intake was compared between the two groups. Generally, the amount of food intake per body weight was larger in the earlier experimental days than that in the later days. Therefore, the mean BAPN intake until day 20 was compared, and there was no significant change between Groups C and D (Table 1).

Blood Pressure

As shown in Figure 4, the mean of the systolic blood pressure of Group A was about 130 mm Hg. In Group B, the administration of DOCA and NaCl was stopped at 7 weeks of age because the general condition became worse. Nevertheless, blood pressure remained more than 180 mm Hg until the end of the experiment. On the

Table 1. *Time of the Onset of the Dissecting Aneurysm and Mean BAPN Intake in Lathyritic Rats without Hypertensive Treatment (Group C) and with Hypertensive Treatment (Group D)*

Case no.	Age at death*	Period from start of BAPN feeding to death (day)		Mean BAPN intake until 20th day (g/kg B.W./day)
Group C				
C-1				0.43
C-2	7w4d	26		0.45
C-3				0.40
C-4				0.40
C-5	8w2d	31		0.42
C-6	9w2d	38		0.44
C-7				0.40
C-8				0.40
C-9	7w5d			0.40
C-10	7w3d	25		0.38
		30.0 ± 5.9		0.41 ± 0.02
Group D				
D-1	5w6d	14		0.38†
D-2	8w2d			0.35
D-3	7w0d	22		0.36
D-4	6w2d	17		0.41†
D-5	8w4d	33	‡	0.39
D-6	7w0d	22		0.41
D-7				0.47
D-8	7w1d	23		0.37
D-9	6w2d	17		0.43†
D-10	6w4d	19		0.41†
		20.9 ± 5.8		0.40 ± 0.04

* Two animals (C-9 and D-2) died of debilitation. The other 12 rats died of dissecting aneurysm.
† The mean BAPN intake until death.
‡ $P < 0.05$ (unpaired t-test).

other hand, the rats fed with BAPN either with (Group D) or without (Group C) hypertensive treatment maintained their blood pressure at lower levels compared with their counterparts. In Group D, the administration of DOCA and NaCl was continued until either death or the end of the experiment, but blood pressure never exceeded 150 mm Hg.

The blood pressure of Group D was higher than that of Group C in the earlier days, but lowered to similar levels to that of Group C in the later days (Figure 4). This result was accounted for by the fact that the rats that demonstrated higher blood pressure in Group D tended to die of dissecting aneurysm in the earlier days, and that

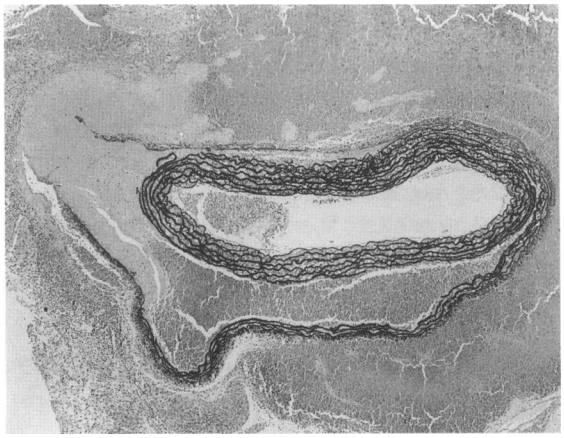

Figure 3. *Dissecting aneurysm of the aorta of the lathyritic rats. Dissection occurred at the outer layer of the media and a rupture and hemorrhage were associated. Elastica van-Gieson stain, magnification ×30.*

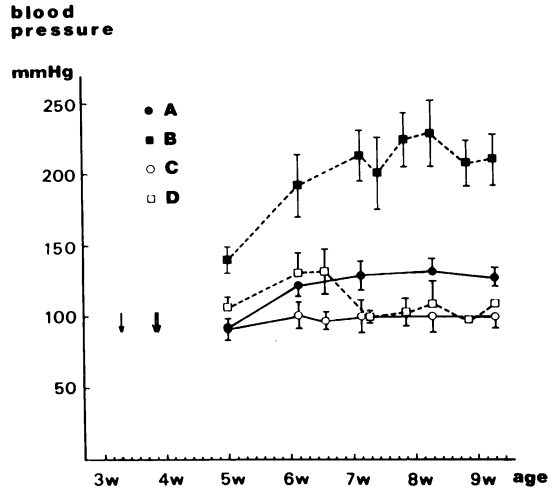

Figure 4. *Time sequential change of systolic blood pressure.* A: *Control rats.* B: *Hypertensive rats.* C: *Lathyritic rats without hypertensive treatment.* D: *Lathyritic rats with hypertensive treatment. The single and double arrows indicate the time of heminephrectomy and the start of BAPN feeding and administration of DOCA and NaCl, respectively, (Mean ± SD).*

the rats that showed lower blood pressure tended to survive longer (Figure 5).

Elastic Architecture of Aorta in Control Rats

In Group A, light microscopic observations demonstrated that the elastin of the ascending aortic media consisted of 10 to 12 layers of elastic laminae and interlaminar elastic fibers present between them. In the section that was parallel to the long axis of the aorta, interlaminar elastic fibers were present among the cross-sectioned smooth muscle cells as if they were arranged radially (Figure 6a). In the section that was vertical to the long axis of the aorta, smooth muscle cells were longitudinally sectioned and interlaminar elastic fibers were present around the smooth muscle cells as if they were arranged along the cells. There was not an excess amount of deposition of GAG or fibrosis. Ultrathin sections stained with tannic acid demonstrated black-stained elastic laminae and interlaminar elastic fibers that were present among the smooth muscle cells (Figure 6b). A higher magnification showed that the irregularly shaped elastic fibers were attached to the processes of smooth muscle cells (Figure 6c). Scanning electron microscopic observations demonstrated a three-dimensional structure of elastin consisting of sheetlike elastic laminae and networks of interlaminar elastic fibers (Figure 6d). Each elastic lamina was interconnected with the networks of the interlaminar elastic fibers and the elastin consequently showed a continuous frameworklike structure.

A morphometric analysis revealed that the area of the interlaminar space occupied by interlaminar elastin was less in the inner media than the outer media in Group A (Table 2).

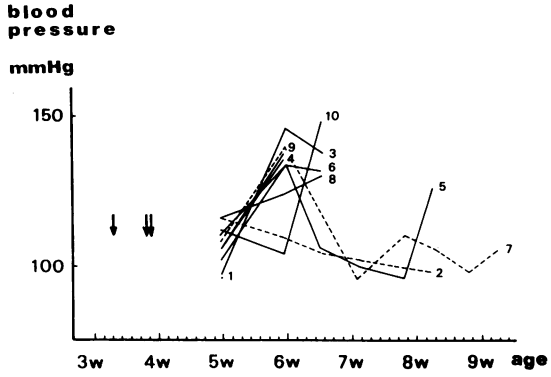

Figure 5. *Time sequential change of the systolic blood pressure of the rats in Group D. Solid lines represent the changes of the blood pressure of the rats that died of dissecting aneurysm. Dotted lines represent the blood pressure of the rat that died of debilitation or was killed at termination of the experiment. The numbers represent the rat numbers indicated in Table 1. The single and double arrows indicate the time of heminephrectomy and the start of BAPN feeding and administration of DOCA and NaCl, respectively.*

Elastic Architecture in Lathyritic Rats without Hypertensive Treatment

A light microscopic examination revealed a prominent reduction of the interlaminar elastic fibers in Group C compared with Group A (Figure 7a). However, no apparent disruption of the elastic laminae was recognized. GAG was diffusely or focally deposited in the interlaminar spaces; however, no large-sized cystic medial necrosis could be observed. These findings were more clearly demonstrated by transmission and scanning electron microscopies. As demonstrated in Figures 7b and 7c, interlaminar elastic fibers were prominently reduced. On the other hand, the elastic laminae were well preserved and smooth muscle cells were regularly arranged in the interlaminar spaces. Morphometric analysis revealed that the area of interlaminar elastic fibers of Group C were significantly reduced both in the inner and outer media as compared with those of Group A (Table 2).

In this group, it took various length of time before fixation for the aorta of the rats that died of dissecting aneurysm or debilitation. The aortas of the other rats were fixed immediately after sacrifice. Light and transmission electron microscopic examinations showed autolytic change of smooth muscle cells and intercellular edema in the former. However, a morphometric analysis did not make significant difference in the area of interlaminar elastic fibers between the rats that died of dissecting aneurysm or debilitation (inner media, 20.4 ± 9.4; outer media, 27.1 ± 10.6, n = 5) and the rats sacrificed at 9 weeks of age (inner media, 19.7 ± 8.3; outer media, 18 ± 8.9, n = 5). The tissue integrity of the kidney and the heart of the former rats were light microscopically almost preserved, except for an autolytic change of renal tubules in mild to moderate degree.

Morphometric Analysis in Perfusion-fixed Aorta

Light and electron microscopic examinations of the perfusion-fixed aortas showed similar findings to those of immersion-fixed aortas, except for more rectilinear elastic laminae and thinner interlaminar spaces. A morphometric analysis revealed a significant reduction of the area of the interlaminar elastic fibers in the BAPN treated rats as compared with that of nontreated rats (Table 3). The extent of the reduction was similar to that of immersion-fixed aortas.

Influence of Impaired Body Weight Gain on Elastogenesis

The body weight of the rats fed with restricted amount of feed became 134.0 ± 7.1 g at 9 weeks of age. The

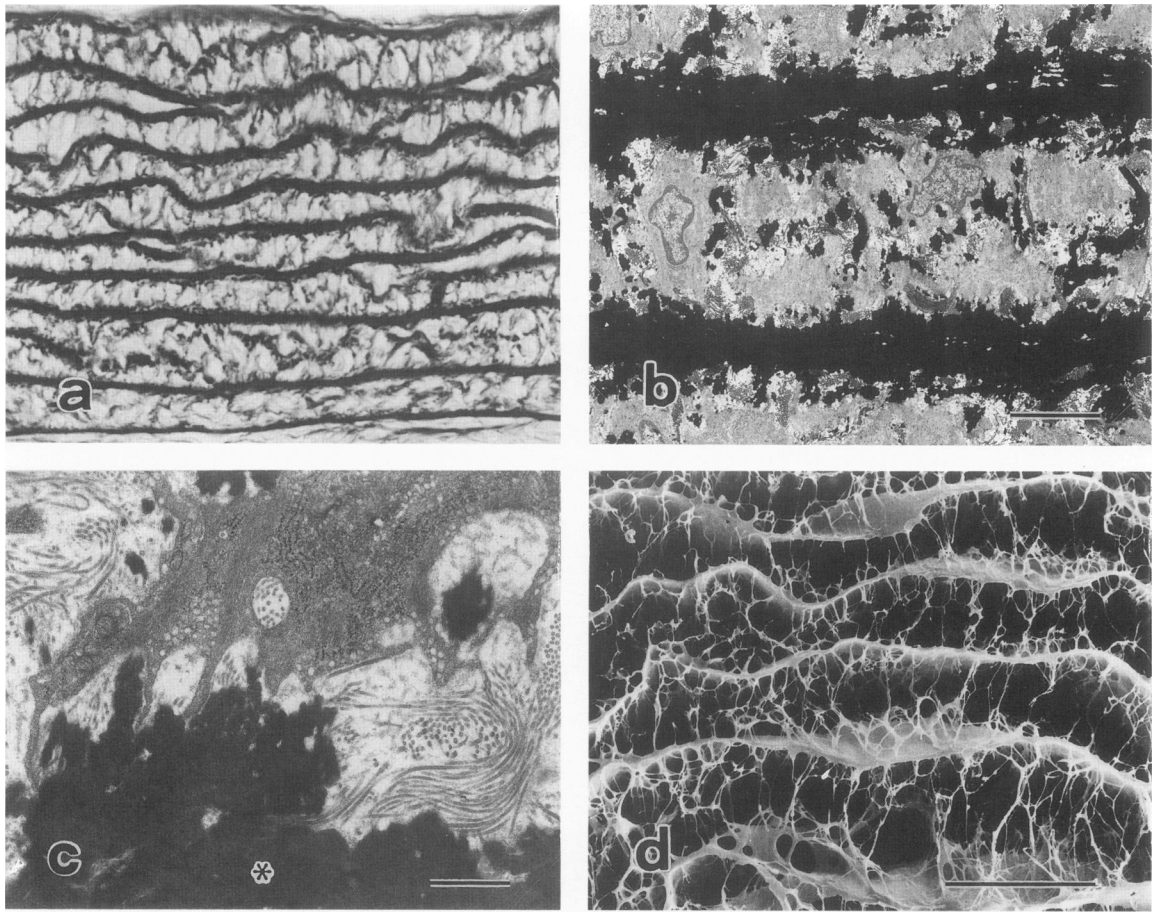

Figure 6. *Elastic architecture of the aortic media of control rats.* **a:** *Light microscopy with elastica van-Gieson stain, magnification ×430.* **b:** *Transmission electron microscopy with tannic acid stain; bar represents 5 μm.* **c:** *Transmission electron microscopy with tannic acid stain superimposed on toluidine blue O stain, (*), elastic lamina; bar represents 1 μm.* **d:** *Scanning electron microscopy after hot formic acid treatment; bar represents 50 μm. All the figures are demonstrated as intimal side up.*

weight was statistically significant from that of the rats that were fed *ad libitum* (Group A, 312.4 ± 28.0 g, $P < 0.001$) but not from that of the rats that were fed with BAPN (Group C, 134.9 ± 10.6 g). However, light and electron microscopic examinations did not reveal any different architecture of aortic elastin between these low body weight rats and the rats in Group A. The areas of the interlaminar elastic fibers of inner and outer media were 76.8 ± 17.9 and 96.2 ± 20.2, respectively, which were not statistically significant from those of Group A.

Elastin Spotted with GAG in BAPN Treated Rats

Toluidine blue 0 stain revealed round- to oval-shaped elastin spotted with GAG in the media of BAPN-treated rats (Figure 8a). It was devoid of surrounding microfibrils and its connection to smooth muscle cells seemed to be much less than that of normal elastin. This kind of elastin was more intensely stained than normal elastin and was easily recognized when ultrathin sections were stained with tannic acid stain superimposed on toluidine blue 0 stain. It was diffusely attached to the elastic laminae and scattered in the interlaminar spaces throughout the entire thickness of the media (Figure 8b).

Table 2. *Morphometric Analysis of Interlaminar Elastic Fibers in Groups A to D*

	Area of interlaminar* elastic fibers (Mean ± SD, N = 10)		
Group	Inner† media		Outer‡ media
A	66.3 ± 13.0	—‖—	90.9 ± 22.6
B	58.5 ± 14.2	—¶—	76.9 ± 10.6
C	20.0 ± 8.4		22.6 ± 10.4
D	18.9 ± 11.1		23.3 ± 15.8

* Area of interlaminar elastic fibers was evaluated by point counting method as described in Materials and Methods.

†‡ One-way analysis revealed statistical significances among the Groups A and D ($P < 0.001$). Comparisons between two groups using modified *t*-statistics were as follows: † A:C ($P < 0.001$); A:D ($P < 0.001$); B:C ($P < 0.001$); B:D ($P < 0.001$).

‡ A:C ($P < 0.001$); A:D ($P < 0.001$); B:C ($P < 0.001$); B:D ($P < 0.001$). Paired *t*-test revealed a statistical significance in both ‖ and ¶ as follows: ‖$P < 0.05$; ¶$P < 0.01$.

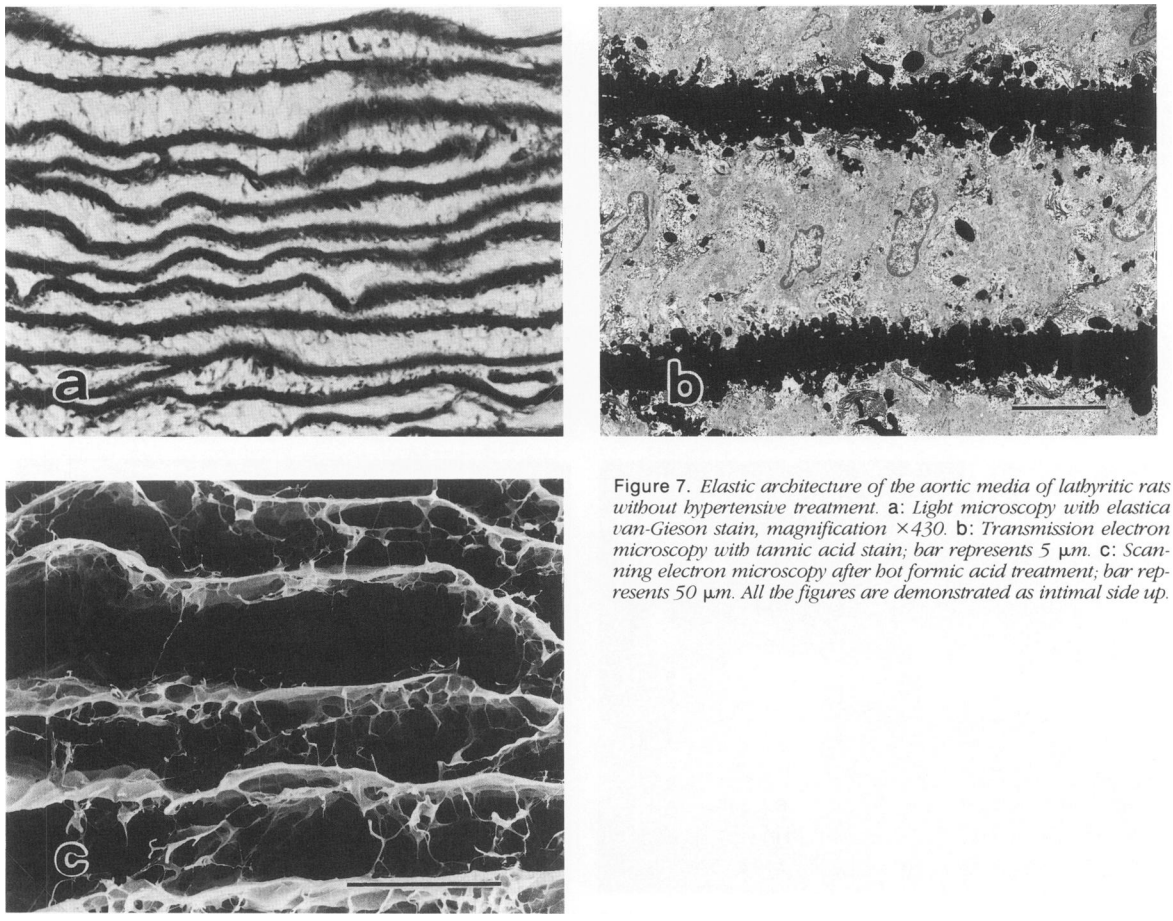

Figure 7. *Elastic architecture of the aortic media of lathyritic rats without hypertensive treatment.* a: *Light microscopy with elastica van-Gieson stain, magnification ×430.* b: *Transmission electron microscopy with tannic acid stain; bar represents 5 μm.* c: *Scanning electron microscopy after hot formic acid treatment; bar represents 50 μm. All the figures are demonstrated as intimal side up.*

Elastic Architecture in Hypertensive Rats and Lathyritic Rats with Hypertensive Treatment

The elastic architecture in Group B was not essentially different from those of Group A under morphologic observations. Scanning electron microscopically, the elastic laminae interconnected each other with the networks of the interlaminar elastic fibers (Figure 9a), and transmission electron microscopically, the elastin was irregularly shaped and connected to smooth muscle cells. There was no round-shaped elastin. Although a morphometric analysis showed a slight reduction of the area of the interlaminar elastic fibers in Group B as compared with those of Group A, it was not statistically significant (Table 2).

The aortas of Group D showed similar findings to those of Group C under morphologic examinations. The interlaminar elastic fibers were prominently reduced (Figure 9b) and GAG was deposited in greater amounts in the media compared with the control rats. A morphometric analysis revealed that the reduction of the area of the interlaminar elastic fibers was at a similar level to those in Group C (Table 2).

Discussion

Elastin is considered to play an important role in stabilizing the aortic media against various forces that act on the wall. Clark and Glagov claimed that the smooth muscle cell to elastic fiber connection was one of the main stabilizing factors of the media.[12,13] The present study demonstrated a three-dimensional continuous structure of elastin in the control rats and each elastic lamina was interconnected by networks of the interlaminar elastic fibers. This finding suggest that this framework-like elastic structure itself also contributes to stabilizing the media. Similar elastic structure was also reported in the Wister-Kyoto rat[7] and in other species, such as humans[5] and rabbits.[14]

Morphologic examinations and morphometric analyses revealed the significant reduction of the interlaminar elastic fibers in the media of the lathyritic rats, whereas elastic laminae were not disrupted and smooth muscle cells were well preserved. These findings were obtained from the rats that died or were sacrificed at different weeks of age and whose aortas were immersion-fixed after different time lapse. Nevertheless, the aforementioned findings were considered to be reliable, because

Table 3. *Morphometric Analysis of Interlaminar Elastic Fibers in Perfusion-fixed Aorta*

BAPN treatment	Area of interlaminar* elastic fibers (Mean ± SD)	
	Inner media	Outer media
non-treated (n = 10)	83.5 ± 12.8 —‖— †	99.4 ± 14.9 ‡
treated (n = 9)	28.7 ± 7.7	27.8 ± 6.1

* Area of interlaminar elastic fibers was evaluated by point counting method as described in Materials and Methods.
† $P < 0.001$.
‡ $P < 0.001$ (unpaired t-test).
‖ $P < 0.05$ (paired t-test).

similar findings were obtained from the perfusion-fixed aortas, although the rats were killed in the earlier experimental days before they died of dissecting aneurysm, and because morphometric comparisons between the aortas that were fixed immediately after sacrifice and the aortas that were fixed after various lengths of time did not show any significant difference. Furthermore, the alteration of the elastic architecture recognized in the lathyritic rats was not considered to be secondary due to physical damage after dissection but primary. This was because the observed areas were apart from the dissected portion and similar alteration was observed in the perfusion-fixed aorta of BAPN-treated rats that were killed before dissecting aneurysm occurred.

Hosoda and Iri also briefly described the diminution of interlaminar elastic fibers in BAPN-treated rats under light microscopic examination.[15] However, electron microscopic examinations in the present study clarified it as a three-dimensional structural alteration of elastin. The finding suggests that the interconnections between each elastic lamina, as well as the interconnection between elastin and smooth muscle cells, are reduced in the lathyritic rats compared with the control rats. These alterations might provoke an unstableness and weakness of the aortic media against various forces, especially the force that dissects the media, and might be related to the initiation and progression of the dissecting aneurysm. A similar idea was recently advanced by Carson and Roach from physiologic viewpoints.[16] They reported that a high non-physiologic hydraulic pressure was needed to initiate the formation of a tear within the tunica media in normal pig aorta and suggested that interlaminar material and fused points of laminae prevented a dissection.

Strikingly similar morphologic findings were recognized in the aorta of the human cases of dissecting aneurysm. Previously, we reported a reduction of the interlaminar elastic fibers in the outer media of dissecting aneurysm of non-Marfan's syndrome,[5] and Perejda et al reported similar findings in the aortic media of Marfan's syndrome.[17] These results suggest that the same mechanism plays a pathogenetic role in the initiation and progression of the dissecting aneurysm independently of species.

Various morphologic alterations were also reported in the aortic media of lathyritic animals, such as fragmentation or degeneration of elastin and increased deposition of ground substance.[18–21] Increase of GAG and decrease of elastin was also demonstrated by biochemical analysis.[22] However, the relation between these morphologic alterations and the pathogenesis of the dissecting aneurysm was not sufficiently discussed in these reports.

The reduction of the interlaminar elastic fibers in the lathyritic rats was considered to be caused by BAPN treatment itself and not by impaired body weight gain, because low body weight rats that were induced by restricted feed intake did not show any different elastic architecture from that of the control rats. BAPN is biochem-

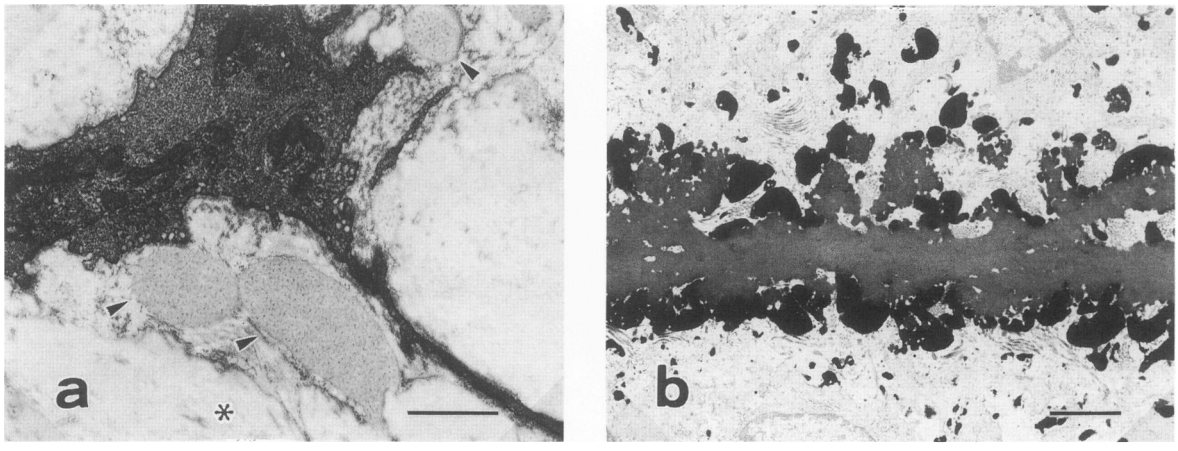

Figure 8. *Elastic architecture of the aortic media of lathyritic rats without hypertensive treatment.* **a**: *Transmission electron microscopy with toluidine blue O stain. Round-shaped elastin was spotted with toluidine blue O-positive GAG (arrowhead); (*), elastic lamina; bar represents 1 μm.* **b**: *Transmission electron microscopy with tannic acid stain superimposed on toluidine blue O stain. The elastin spotted with GAG was deeply stained; bar represents 2 μm.*

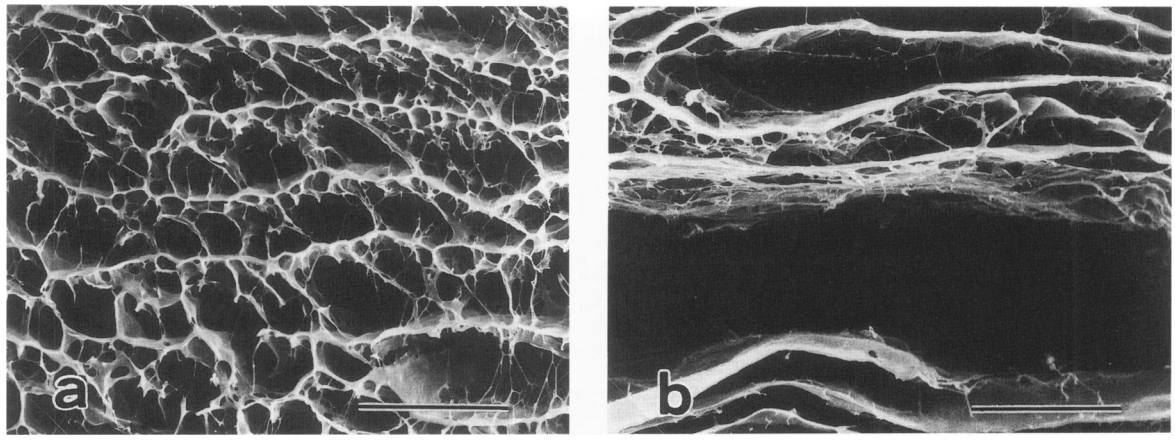

Figure 9. *Elastic architecture of the aortic media of hypertensive rats and lathyritic rats with hypertensive treatment. Scanning electron microscopy after hot formic acid treatment.* **a** *Hypertensive rat.* **b**: *Lathyritic rat with hypertensive treatment. Large space was a dissected lamina; bars represent 50 μm. Both figures are demonstrated as intimal side up.*

ically reported to be an inhibitor of lysyl oxidase and inhibits the crosslinking of elastin,[23,24] and thus, may have disturbed the elastogenesis of interlaminar elastic fibers observed in the lathyritic rats. The disturbance of elastogenesis is also suggested by the round-shaped elastin spotted with GAG, which was previously reported by Fornieri et al.[10] They claimed that it was not a mature elastin but an immature one, in which the deamination of lysine epsilon amino groups was inhibited by BAPN.

BAPN also inhibits the crosslinking of the collagen by inhibiting lysyl oxidase activity.[23,24] A reduction in the rigidity of collagen fibers may cause a laxity of the aortic media, and might have contributed to some alterations of the elastic architecture observed in the present study. The disturbance of collagen metabolism may also have been related to the lower blood pressure recognized in the lathyritic rats with or without hypertensive treatment compared with nonlathyritic counterparts. Similar findings were reported in the BAPN treated rats by other investigators.[25,26] They also reported the reduction of lysyl oxidase activity in the arterial wall and suggested that inhibition of formation of crosslinked collagen reduces the vascular rigidity and prevents a rise of blood pressure.

The positive association between hypertension and dissecting aneurysm is a well known fact in human cases[3] as well as in the turkey's spontaneous onset model.[27] The present results suggest that the onset of dissecting aneurysm was accelerated by the rise of blood pressure in the lathyritic rats. Rise of blood pressure might play two possible roles on the arterial wall in the lathyritic rats: changing the structure of the wall and acting as a strong physical force against the wall that became unstable by BAPN treatment. The present study suggest the latter possibility, because the dissection tended to occur soon after the rise of blood pressure, and because no different architectural change of elastin was detected between the lathyritic rats with hypertensive treatment and those without treatment. Furthermore, this may be supported by the fact that the high systolic blood pressure in Group B did not make any significant change in the elastic architecture and other components as compared with those of the control rats. However, we should be careful since short-term hypertensive state induced in the present study is greatly different from that of human cases that usually show a long-term duration. In the animal model, long-term hypertension is reported to provoke a focal necrosis of smooth muscle cells and a diminution of elastin in the rat aorta.[28] On the other hand, short-term hypertension causes hypertrophy of the aortic media and an increase of elastin content.[29–32] Further studies are needed to clarify the relation of hypertension to dissecting aneurysm.

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