◆ EXPERIMENTAL INVESTIGATION ◆

Early Histological Changes in the Porcine Aortic Media After Thoracic Stent-Graft Implantation

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Purpose: To describe the histological findings in the aortic wall 5 days after thoracic endovascular aortic repair (TEVAR) in a porcine model.

Methods: Two overlapping stent-grafts were implanted in each of 6 juvenile pigs, covering the entire descending thoracic aorta (DTA). On the 5th postoperative day, tissue samples were taken from the DTA in each animal. Medial thickness and medial necrosis were quantified and compared to measurements from the aortas of 6 control animals.

Results: Significant medial thinning was observed in stent-covered regions in the test animals. At the proximal landing zone, aortic wall thickness changed from 1387 ± 68 to 782 ± 74 μm within the covered aortic segment ($p=0.028$); at the distal landing site, the wall thickness was 365 ± 67 μm within the stent and 501 ± 57 μm distally ($p=0.028$). In the overlap zone, the aortic wall measured 524 ± 122 vs. 1053 ± 77 μm in native controls ($p=0.004$). Aortic thickness proximal to the graft did not differ from the proximal region of native aortas (1468 ± 96 vs. 1513 ± 80 μm , $p=0.423$), but the aorta was significantly thinner distal to the stent (707 ± 38 vs. 815 ± 52 μm , $p=0.004$). Laminar necrosis constituted $38\% \pm 7\%$ of the media in the proximal landing zone, $54\% \pm 4\%$ in the overlap zone, and $46\% \pm 13\%$ in the distal landing zone.

Conclusion: In this porcine model, significant medial thinning and necrosis of the stented aorta was observed. The findings suggest an early phase of vulnerability of the aortic wall, before scarring and adaptive changes have strengthened the residual aorta.

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Key words: thoracic endovascular aortic repair, blood vessel prosthesis, endovascular repair, aortic wall histology, animal model

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In recent years, endovascular repair has emerged as an appealing alternative to open surgery for various thoracic aortic pathologies.¹ Current data suggest that the perioperative mortality and morbidity associated with thoracic stent-grafting is lower than with traditional open repair.² Nevertheless, reintervention

rates remain quite high after thoracic endovascular aortic repair (TEVAR), and late endograft-related complications requiring open conversion are rare but accompanied by substantial morbidity.^{3,4}

Contemporary stent-grafts are held in place by radial forces emanating from a wire scaffold

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TABLE

Aortic Diameter and Stent-Graft Oversizing at
Specific Regions of the DTA

	Aortic Diameter, mm*	Oversizing
Proximal	13.7±0.2	17%
Mid thoracic	12.1±0.2	20%
Distal	9.6 ±0.3	25%

Continuous data are presented as the means ± standard deviation.

* Fluoroscopic measurement of 5 pilot animals 2 cm distal to the subclavian artery (proximal diameter), in the mid thoracic aorta (10 cm proximal from the celiac axis), and 1 cm proximal to the celiac axis (distal diameter).

(nitinol or stainless steel). Additionally, the devices may be equipped with (covered/uncovered) springs or barbs, enforcing endoluminal fixation. Systemic inflammatory conditions after TEVAR, known as post-implantation syndrome, have been described.⁵ However, data on local aortic wall alterations after stent-grafting, such as the integrity of the aortic wall, are limited. The goal of this experimental animal study was to describe histological changes occurring in the aortic wall in the early phase after TEVAR.

METHODS

Study Design

In this prospectively designed study, aortic wall tissue of 6 Yorkshire pigs weighing a mean 27.1±0.7 kg (Animal Biotech Industries Inc, Danbora, PA, USA) in which thoracic stent-grafts were implanted in consecutive operations was compared to corresponding aortic material from 6 native control animals (27.0±0.8 kg) to investigate aortic wall alterations after TEVAR. The experiment was embedded in a study on spinal cord injury previously published by our group,⁶ which involved a strict 5-day protocol. The entire thoracoabdominal aorta was removed in a necropsy 5 days after stent-graft implantation, and aortic tissue samples were processed for histopathological analysis.

All animals received humane care in accordance with the guidelines from Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory

Animals published by the National Institutes of Health. The Mount Sinai Institutional Animal Care and Use Committee reviewed and approved the protocol for this research.

Thoracic Stenting

Perioperative management and anesthesia have previously been described in detail.⁶ Before initiation of the experiments, angiographic measurements of the aortic diameter were conducted in animals of the same weight and size by using a pigtail catheter with radiopaque markers as a reference standard to find the best stent conformation (Table). Due to the specific size and diameter of the descending thoracic aorta (DTA) in this porcine model, the Excluder AAA Endoprosthesis (W.L. Gore & Associates, Inc., Flagstaff, AZ, USA) was selected.

In every animal, 2 stents were placed, overlapping ~2 cm. To prevent migration at deployment, the stents were oversized ~15% to 25%. The area covered by the stent extended from 2 to 3 cm distal to the subclavian artery to 1 cm proximal to the celiac axis. Once access was established, an atraumatic pigtail catheter was inserted, and an initial angiogram was performed to locate the level of the celiac axis and the proximal landing zone. The celiac axis was evaluated, and the vessel was marked and displayed on a separate screen. Subsequently, a guidewire was placed up to the aortic arch, over which the distal stent (catalogue number PXC121200; 12 cm long) was inserted and advanced under fluoroscopic guidance. The distal end of the stent was placed just above the celiac axis. After deployment, the stent was modeled with a balloon catheter to ensure optimal apposition to the aortic wall. The second stent (PXC141400; 14 cm long) was deployed so as not to impinge on the subclavian artery but adequately overlap the distal stent. Similarly, careful balloon dilation of the stent-graft was performed to guarantee full expansion of the device. A final postoperative aortogram was performed to confirm the appropriate positioning of the stent-grafts in the DTA.

Histological Analysis

On the 5th postoperative day, the animals underwent another angiogram to detect any

Figure 1 ♦ Fluoroscopy showing tissue removal sites for stented aortas proximally, distally, and in the overlap zone. Medial thickness and medial necrosis were evaluated on a horizontal specimen. At the proximal and distal junctions, a longitudinal sample was taken, and both the non-stented and the stented areas were evaluated.

complications and to ensure stent apposition to the entire wall with no perigraft blood flow. After systemic heparinization, the animals were euthanized with an anesthetic overdose of 120 mg/kg of pentobarbital, and the entire thoracoabdominal aorta was removed during the ensuing necropsy.

After 7 days of formalin fixation, the aorta was opened longitudinally, and the 2 stents were carefully peeled off the aortic wall. For histopathological analysis, 5 tissue samples were cut from each aorta (Fig. 1): a sample 1 cm proximal to the stent, another one 1 cm distal to the stent, a sample from the area in which the 2 stents overlapped, and finally samples at the proximal and distal landing zones, where the non-stented aorta transitions into the stented part and vice versa (proximal/distal transition zone). Here a longer specimen was prepared, and both ends were analyzed and compared. In the 6 untreated control animals, the harvested aortas were sampled at corresponding levels: a proximal sample, 2 cm distal to the subclavian artery, a sample in the midthorax, and a distal sample just above the celiac axis.

After sampling, the aortic tissue was immediately embedded in paraffin and subsequently

cut and stained in hematoxylin-eosin (H&E) and van Gieson elastica. Medial thickness was quantified by obtaining digital images with a light microscope (Zeiss Axioplan2IE and Zeiss AxioCam MRc cameras; Carl Zeiss Microlmaging GmbH, Jena, Germany) and measuring the distance between the internal and external elastic laminae in van Gieson-stained segments. In areas where the media showed a sinusoidal pattern caused by indentations of the stent struts, the thickness was measured in places of compression and clear of them, and the mean value was determined. H&E-stained specimens were used for analysis of inflammation and for quantification of medial necrosis.

Statistical Analysis

Data were entered in spreadsheets (Microsoft Excel for PC, 2003; Microsoft, Redmond, WA, USA) and transferred to PASW Statistics (version 18.0; IBM Corporation, Somers, NY, USA) for description and analysis. Continuous data are presented as the means $\pm$ standard deviation; categorical data are given as the counts (percentage). The Wilcoxon test was used for analyzing dependent variables and the exact or Kruskal-Wallis test for independent variables. $P < 0.05$ was considered to indicate statistical significance.

RESULTS

Technical success of stent deployment was achieved in every animal. The stent-graft was oversized by 17% proximally and by 25% distally and extended from 2 to 3 cm distal to the subclavian artery to 1 cm proximal to the celiac axis, covering an aortic segment 20.2 ± 1 cm in length. During the postoperative angiogram, the intended treatment length was confirmed, and no endoleak or instances of stent migration/collapse were observed. None of these complications arose during the subsequent 5 days, as demonstrated by a second angiogram before sacrifice.

Histopathology

At the time of necropsy, open exploration of the descending thoracoabdominal aorta of the test animals found gross aortic wall

Figure 2 ♦ Luminal medial necrosis in the overlap region (hematoxylin and eosin; original magnification $\times 40$). Note the loss of nuclei in the luminal smooth muscle cells. The * indicates the measure of medial necrosis (258 μm); the # indicates the measure of medial thickness (489 μm).

thinning in areas corresponding to those that had been covered by the stent-grafts.

Histology of the aorta on control animals showed that the aortic wall is composed of 3 layers similar to what is observed in humans. The intima consists of a single layer of endothelial cells; the media is bounded by internal and external elastic lamina and is composed mainly of smooth muscle cells and concentrically arranged layers of elastic and collagen fibers. The adventitia consists of connective tissue containing the vasa vasorum and nerves. The findings in the non-stented aortic segments of the experimental animals were the same as in these 6 controls.

In all tissue samples in which the stent covered the aorta, medial necrosis was present in the luminal half of the wall. Laminar necrosis constituted $38\% \pm 7\%$ of the media in the proximal landing zone, $54\% \pm 4\%$ in the overlap zone, and $46\% \pm 13\%$ in the distal landing zone (Fig. 2). There was a significant increase in necrosis when the overlap zone was compared with the proximal landing zone ($p=0.028$), but no significant difference was found between the overlap and the distal landing zones ($p>0.05$).

Substantial differences were seen when comparing medial thickness of non-stented segments to stented aortic wall specimens

(Fig. 3). Significant medial thinning was observed in all stent-covered regions. At the proximal landing zone, aortic wall thickness changed from 1387 ± 68 to 782 ± 74 μm within the covered aortic segment ($p=0.028$); at the distal landing site, the wall thickness was 365 ± 67 μm within the stent and 501 ± 57 μm distally ($p=0.028$). In the overlap zone, the aortic wall measured 524 ± 122 vs. 1053 ± 77 μm in native controls ($p=0.004$). Aortic thickness proximal to the graft did not differ from the proximal region of native aortas (1468 ± 96 vs. 1513 ± 80 μm , $p=0.423$), but the aorta was significantly thinner distal to the stent (707 ± 38 vs. 815 ± 52 μm , $p=0.004$).

In all stent-covered regions, the metal stent struts left indentations in the aortic wall. Between those indentations, thrombus formation gave the luminal surface a sinusoidal pattern (Fig. 3B). The elastic and collagen fibers were stretched and had lost their wave-like structure, but were not fragmented. In 5 of 6 aortas, inflammatory infiltration was seen within the media; in one, an early intimal foreign body reaction with multinucleated giant cells was evident. No inflammatory infiltrate was seen either proximal or distal to the stent.

DISCUSSION

Since its introduction in 1994, TEVAR has continued to evolve and has produced favorable results for patients with various thoracic aortic pathologies.^{1,7} However, data are limited on the interaction between the stent-graft and the aortic wall with regard to biomechanics and inflammatory and structural changes.^{8–13} In this study, we analyzed histological alterations 5 days after TEVAR in a porcine model and found significant aortic wall thinning and luminal necrosis in every specimen covered by the stent.

As in humans, nutrition of the DTA wall in the pig is thought to be provided both by the vasa vasorum and via endoluminal diffusion.^{14,15} In TEVAR, both nutritional pathways are interrupted at once, since endovascular stent-grafting leads to complete coverage of the luminal wall by graft material. Concomitant occlusion of the segmental arteries leads to diminished blood flow within the vasa

Figure 3 ♦ (A) Microscopic picture of the non-stented distal transition zone (van Gieson elastica stain, original magnification $\times 20$). Medial thickness: 454 μm . (B) Microscopic picture of the stented distal transition zone (van Gieson elastica stain, original magnification $\times 20$). Note the thinned compressed media and thrombus formation to either side of the stent-strut indentation. Medial thickness at indentation: 316 μm .

vasorum of the DTA, which arise from the segmental arteries within the periadventitial fatty tissue.

Intercostal artery ligation and its consequence for the nourishment of the aortic media have been studied by Heistad et al.¹⁶ in a dog model. The authors describe morphological changes ranging from broad bands of necrosis to patchy areas of cell loss, primarily in the middle layers of the aortic media. The inner third of the aortic wall showed normal morphological architecture after segmental artery ligation, which suggests that endoluminal diffusion of nutrients is sufficient to nourish this segment of the aorta. In a study conducted by Stefanadis et al.,¹⁷ the periaortic fat that contains the vasa vasorum for the ascending aorta was removed in 7 dogs. In all animals, a uniform band of necrosis of the outer layers of the media developed in the region that had been supplied by the removed periaortic vasa vasorum network.

We observed medial necrosis in all stent-grafted aortas affecting up to 50% of the luminal half of the wall 5 days after TEVAR. Significant luminal necrosis may suggest that the disturbance of the luminal nutritional pathway outweighs in importance the disruption of blood flow through the vasa vasorum in the setting of thoracic stent-grafting. In addition to medial necrosis, significant thinning of

the media was found in all stent-covered regions, notably in the proximal and distal landing zones. In aneurysm repair, endograft fixation ultimately depends upon the radial force of the scaffold wire, which holds the graft in place. In the experimental model of Rousseau et al.,⁸ medial necrosis due to high mural pressure was described in stent-covered areas. We cannot be sure whether our finding of luminal medial necrosis is due to pressure, ischemia, or both. The observation that necrosis in the proximal landing zone was significantly less severe (stent oversizing: 17%; necrosis: 38%) than damage in the distal landing zone (stent oversizing: 25%; necrosis 46%) may suggest at least some impact of pressure. Additionally, we observed that medial necrosis was most severe in the overlap region, even though the stent-grafts were more markedly oversized distally (overlap region: stent oversizing, 20%; necrosis, 54%). A study that investigated the mechanical properties of endovascular stent-grafts found that overlapping 2 stents increased radial force to values greater than the sum of those parameters for the individual stents,⁹ which may explain our finding the most severe necrosis in the region where the stents overlapped and underlines the likely role of pressure in the pathogenesis of medial necrosis. An interesting method for stent-graft fixation with fibrin glue was investigated by

Almeida et al.,¹² who concluded that fibrin glue-impregnated endoprostheses may promote better fixation. This approach may be a promising alternative to oversizing, since it reduces the radial force exerted on the aorta.

Siegenthaler et al.¹⁰ performed TEVAR in a porcine model in order to evaluate aortic growth patterns and morphology after thoracic stent-graft placement. Explantation of the graft was undertaken after an interval of 6 to 15 months. In contrast to our findings, the histology of the stented aorta revealed significant thickening of the media. However, there was segmental fibrosis of the inner one third of the media, with loss of smooth muscle cells and compression of the elastic fibers. The outer two thirds of the media showed normal architecture. It has been postulated that mechanical stretching, endothelial injury, and pressure may cause smooth muscle cell mitogenesis and migration.¹⁸ The activated cells produce a fibrillar or collagenous matrix in the media as a healing response.¹³ In this study, we may be observing an early phase of medial destruction before the fibrous remodeling of the wall takes place and strengthens the residual aorta.

The stent-grafts used in our animals were oversized by 15% to 25%. Initial device selection was based on angiographic studies and post mortem measurements undertaken during the pilot phase of the study. Histological evaluation of the specimens covered by the stent showed stretching of the elastic and collagen fibers, which is a structural correlate of strain. A recent animal study conducted by Sincos et al.¹¹ performed 2 biomechanical tests to evaluate the effect of different levels of stent-graft oversizing (up to 40%) on non-atherosclerotic aortas 4 weeks after implantation. Maximum supported strength, maximum tension, and stress of the aortic wall all were reduced proportional to the degree of oversizing in all groups. During a detachment test, which measured the strength necessary to remove the stent-graft from the aorta, this weakening of the wall was confirmed by the rupture of 2 samples when oversizing of >30% was applied. The authors concluded that the biomechanical tests showed that the aortic wall undergoes structural changes after stent implantation, which might be of relevance for patients undergoing TEVAR for

traumatic thoracic injuries. Unfortunately, no histological studies were possible.

A study by Schlosser et al.¹⁹ investigated the causes and timing of rupture after EVAR in 270 patients; 38 ruptures occurred in the perioperative period: 13 during the initial procedure and 25 after the procedure but within 30 days. In 10 of the 25 aneurysms that ruptured after EVAR within 30 days, the aneurysm was not completely excluded after the initial procedure, but in the 15 remaining patients no explanation for early rupture was apparent. We hypothesize that in some of those cases, stent-induced aortic wall degeneration may have occurred after initial aneurysm exclusion, possibly leading to proximal neck dilatation and subsequently to type I endoleaks or stent migration, which are the leading causes of rupture after EVAR.¹⁹

In patients with connective tissue disease, high rates of endoleaks and reinterventions after TEVAR are reported.²⁰ In this cohort of patients, where a certain wall weakness exists *eo ipso*, early structural wall alterations after TEVAR (i.e., within landing zones) may be especially unfavorable. Additionally, we hypothesize that if open repair after TEVAR becomes necessary during the first days after stent implantation, the weakened aortic wall may make a stable anastomosis difficult.

As previously mentioned, the stented aortic tissue was harvested from animals in which we were investigating spinal cord injury by simulating hybrid thoracoabdominal aortic aneurysm repair. All segmental arteries arising from the aorta were occluded in the experiment by open lumbar clamping in addition to endovascular thoracic stenting. During the initial necropsy studies, we observed that the stented aortic wall of the DTA looked macroscopically thinner than the adjacent regions or native aortic tissue; however, we did not begin to systematically investigate this phenomenon until we had 6 consecutive animals in which to do our aortic tissue analysis. Due to the strict protocol of the aforementioned study, all animals were euthanized on the 5th postoperative day, so we could examine only tissue alterations at that specific time point. The findings described must therefore be understood as a snapshot in a developing process. Nevertheless, despite its limitations, the current study is the first to

describe the early phases of aortic wall alterations after TEVAR. However, the number of animals was small, and all experiments were conducted in a nonatherosclerotic porcine model. To what extent these findings might be different in a hypertensive, hypercholesterolemic patient with severe atherosclerosis is a matter of speculation.

Conclusion

Our findings of medial thinning and luminal medial necrosis in areas covered by the stent-graft 5 days after thoracic endografting suggest an early phase of vulnerability to rupture before scarring and adaptive changes can strengthen the residual aorta. The data presented may be valuable to better understand endograft–aortic wall interaction and possibly enhance device development.

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