Chemical-Induced, Nonlethal, Developmental Model of Dissecting Aortic Aneurysm

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BACKGROUND: A chemical-induced, nonlethal, dissecting aortic aneurysm (DAA) is described following in utero exposure to semicarbazide, an inhibitor of the vascular enzyme semicarbazide sensitive amine oxidase (SSAO). **METHODS:** Sprague-Dawley rat dams were given semicarbazide (0.096–49.000 mg/kg/day) by IP injection on gestation days (GDs) 14–20, a period of rapid aortic development. Newborn rats (day 1) were killed and their thoracic organs were removed en bloc for near-serial cross sections and routine histopathology, Movat stain for elastin, and immunohistochemistry to differentiate cells involved in the evolution of the DAA. In subsequent experiments, pups from treated dams (0.096–6.125 mg/kg/day) were allowed to survive for 7 or 28 days. **RESULTS:** DAA occurred in nearly 100% of the rats at all doses except the lowest tested (1.530, 0.096 mg/kg/day). Dissections frequently extended to the carotids and, less frequently, to the abdominal aorta. Remodeling of vascular lesions proceeded by organization of collections of blood in vascular media (the "false lumen"), proliferation of vascular smooth muscle cells, fibrosis, and formation of irregular frayed elastic lamellae in healed vascular media. Biochemical quantitation and Western blot analysis of main extracellular matrix proteins on GD 20 showed no overt difference in expression of collagen type I, fibrillin-1, or elastin. **CONCLUSION:** This developmental model provides investigators an opportunity to explore the pathologic mechanisms of DAA and to examine the potential long-term effects of vascular remodeling of DAA. *Birth Defects Research (Part A) 76:29–38, 2006.* © 2006 Wiley-Liss, Inc.

INTRODUCTION

The term "dissecting aneurysm" refers to the sudden, dramatic tearing or splitting of the medial layers of an artery, which results in false lumens, occlusion of distal vessels (such as coronary arteries), rupture, hemorrhage, and rapid or sudden death (Hagan et al., 2000; Januzzi et al., 2004). It has long been recognized that certain connective-tissue metabolic disorders, most notably Marfan syndrome, carry a high risk for the development of dissecting aneurysms. However, recognition of the occurrence of dissection as an isolated disease, and concern about this lethal vascular disease, which frequently runs in families and affects younger persons, is growing (Helliker and Burton, 2003).

The largest elastic artery, the aorta, is by far the most common site for dissection, and dissecting aortic aneurysm (DAA) almost universally involves the thoracic aorta, with localization in the aortic arch and frequent direct extension to arteries of the head, neck, and upper extremities (Nienaber and Eagle, 2003). Little is known about the mechanism behind the formation of DAA; however, as alluded to above, it is a frequent cause of death in patients with Marfan syndrome, and surgical therapeutic strategies have been successfully devised for patients with this devastating disease. Although several animal models are currently available and are used in the study of abdominal aortic aneurysm (Stehbens, 1999; Manning et al., 2002), no reproducible, nonlethal experimental model exists for the study of isolated DAA.

Mounting evidence suggests that degenerative changes in the structural components of the vascular wall are precursors to dissection (Brooke et al., 2003). Degenerative changes in the media are thought to involve the complex elastin lamellae. These intricately interconnected layers are a complex mix of the large protein, elastin, laid down on a substrate of the microfibrillar protein, fibrillin (Brooke et

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al., 2003). The occurrence of DAA in elderly individuals with hypertension (Angouras et al., 2000) suggests that age-related loss and degeneration of elastin, with consequent weakening in normal elastic properties, may predispose to dissection. Other possibilities include alterations in any of the many complex interstitial proteins, including the collagens.

Recently the effects of in utero exposure on postnatal development (Mericskay et al., 2005) have attracted much interest. Our previous study in weanling rats suggested that inhibition of the enzyme semicarbazide sensitive amine oxidase (SSAO) has a severe deleterious effect on normal vascular development, especially of the aorta (Langford et al., 1999). A recent microarray study revealed that the SSAO homologue, the membrane copper amine oxidase or vascular adhesion protein-1 (VAP-1), is downregulated in human dissecting ascending aortic aneurysms (Muller et al., 2002).

Stimulated by these previous studies, we sought to extend our knowledge about the deleterious effects of SSAO inhibition on vascular development to potential in utero effects. In the present study, by administering the known SSAO inhibitor semicarbazide to timed pregnant rats during the critical period of aortic development, we developed a reproducible, nonlethal model of DAA with striking morphologic similarities to the disease in humans. These initial biochemical and immunohistochemical studies implicate aberrant collagen and/or elastin metabolism as an underlying mechanism of DAA. This model of in utero exposure to relatively nontoxic levels of a xenobiotic can be used to explore how underlying embryologic changes in the aortic wall might lead to the vascular pathology known as dissection. Also, these studies serve to raise awareness about the possibility that environmental factors may adversely affect blood vessels through prenatal influences.

MATERIALS AND METHODS

Chemicals and Animal Treatment

The semicarbazide hydrochloride and all other chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO). Timed pregnant Sprague-Dawley rat dams were treated with either physiological saline (control, $n = 7$) or SSAO inhibitor semicarbazide (at 0.096 [$n = 2$], 1.530 [$n = 2$], 6.125 [$n = 10$], 12.250 [$n = 2$], 24.500 [$n = 2$], and 49.000 [$n = 2$] mg/kg) by IP injection (2.5 ml/kg body weight) daily on gestation days (GDs) 14–20. These experiments were approved by the Animal Experimentation Committee of the University of Texas Medical Branch (protocol 8812178). Doses were based on previously published data regarding the in vivo inhibition of SSAO (Langford et al., 1999).

In initial experiments for each treatment group, newborn rats were killed on day 1 of life (postnatal day [PND] 1) and their thoracic organs were removed en bloc. The litters from dams treated with saline only for the same period of daily injections served as controls throughout these experiments.

In subsequent experiments, pups from dams treated with a more limited dose range of semicarbazide (0.096–6.125 mg/kg) were allowed to survive to PND 7 ($n = 24$ pups from 9 dams) or 28 ($n = 38$ pups from 9 dams).

Morphology Study

Newborn rats (PNDs 1–28) for morphologic examination were killed by carbon dioxide inhalation. Intrapleural and intraperitoneal injections of 1, 2, and 3 ml of 10% neutral-buffered formalin were performed immediately, and the thoracic and abdominal organs were removed en bloc and immersion-fixed in 10% neutral-buffered formalin.

Following routine dehydration and routine processing, 3 levels were step-sectioned at 1) the aortic root at the level of the aortic valve, 2) the midaortic arch, and 3) the thoracic carotid artery. This near-serial cross sectioning allowed for examination of the entire aortic arch and branches. In all but the postpartum day (PPD) 1 group, the abdominal aorta was also sectioned at the level of the renal artery. Histological sections were stained with the use of routine hematoxylin and eosin (H-E), Movat (for collagen and elastin), and Prussian blue (for iron) methods.

Immunohistochemistry

Immunohistochemical staining was used to differentiate cells involved in the evolution or resolution of the DAAs observed, especially with respect to formation of the false lumen (vs. the true aortic lumen). Deparaffinized and rehydrated sections were incubated for 30 min with anti-CD31 (dilution: 1:5; Serotec, Oxford, UK) and anti-α smooth muscle cell (SMC) actin monoclonal antibodies (dilution: 1:300; Dako Cytomation, Carpinteria, CA) followed by staining by the labeled streptavidin-biotin method with a Dako LSAB2 kit (KO684). DAB chromogen (S300; Dako Cytomation) solution was used as the chromogen, and counterstaining was performed with methyl-green. Negative controls consisted of samples in which the primary antibody was omitted.

Collagen and Elastin Quantification

In an additional experiment, total aortic elastin and collagen were measured in 98 fetuses (pooled into 8 groups from 8 pregnant rats), killed at GD 20 (estimated day before birth) from pregnant rats treated with 6.125 mg/kg semicarbazide daily (59 fetuses from 5 treated pregnant rats, and 39 fetuses from 3 control pregnant rats) according to standard methods (Hoffman et al., 1972). The aortas were dissected free of extraneous tissue, rinsed with distilled water, blotted dry, weighed, minced, and extracted with a 3:1 volume ratio mixture of ethanol-ether. The samples were dried under nitrogen and vacuum-desiccated, and dry fat-free weights were determined. Tissues were extracted multiple times with 0.5 M NaCl and 0.5 M acetic acid, and insoluble pellets were lyophilized. Lyophilized pellets were weighed to obtain a dry, fat-free weight. After 5 ml of 0.1 N NaOH was added to each lyophilized sample, were heated in a water bath (98°C for 50 samples/min). The supernatant was collected and combined with the supernatants from 3 additional washings (twice with 2.5 ml, 98°C, 0.1 N NaOH, and once with 98°C hot deionized water). The supernatants were dialyzed for 24 hr against deionized water, frozen in 50-ml tubes, lyophilized, and weighed (yielding the collagen fraction weight). The insoluble elastin pellets were combined with 1 ml of deionized water and then lyophilized and weighed (yielding elastin fraction weight).

Table 1
Dissecting Aortic Aneurysm in Newborn Rat Pups from Dams Treated with Semicarbazide

Dose: mg/kg (n = pups/dams)	Thoracic aorta (%)	Branches of thoracic arch involved (%)	Necrosis in the vessel wall (%)	Tearing entry to the aneurysm (%)	Pulmonary artery hemorrhage (%)
Control (11/3)	0	0	0	0	0
0.096 (8/2)	0	0	0	0	0
1.153 (8/2)	25	0	0	0	0
6.125 (12/3)	91.7	25	58.3	16.7[a]	0
12.25 (12/3)	100	100	91.7	16.7[a]	0
24.5 (12/3)	100	100	100	25	0
49 (10/2)	100	100	90	30	20

[a]1 dam's litter had no abnormal findings.

Western Blot Analysis

In an additional experiment the aortic expression of collagen type I, elastin, and fibrillin-1 (the protein frequently defectively expressed in Marfan syndrome) was analyzed by Western blot in 84 fetuses (pooled into 8 groups from 8 pregnant rats), killed at GD 20 (estimated day before birth) from pregnant rats treated with 6.125 mg/kg semicarbazide daily (40 fetuses from 4 treated pregnant rats, and 44 fetuses from 4 control pregnant rats) according to standard methods (Yang et al., 2004). The aortas were dissected free of extraneous tissue as described above, and the samples (~0.05 g) were homogenized in 0.2 ml of cold buffer solution containing 50 mmol/L Tris-HCl, pH 7.4, and 1.0 mmol/L dithiothreitol and protease inhibitors. The samples were centrifuged and the protein concentration was determined before equal amounts of soluble protein (50 μg/lane) were resolved by electrophoresis on 4–12% NuPAGE Bis-Tris Novex gradient gels (Invitrogen, Carlsbad, CA). The membranes were incubated with goat anti-collagen type I (dilution 1:1000; SouthernBiotech, Birmingham, AL), rabbit anti-elastin rabbit polyclonal antibody (dilution: 1:500; Cedarlane Laboratories, Hornsby, Canada), or goat anti-fibrillin-1 (dilution: 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA) for 90 min, respectively, followed by incubation with a secondary antibody at 1:2,000 for 45 min. The results were quantified using a densitometric imaging system (Alpha Innotech Corp., San Leandro, CA). A Western blot for β-actin was used to verify equal loading and transfer.

Statistical Analysis

The results are expressed as means ± SD. The data were analyzed with unpaired tests or analysis of variance (ANOVA) as appropriate. Statistical significance was assumed at $P < .05$.

RESULTS

General Observations

No differences in maternal weight, stillbirths, litter size, or mean pup weight were found among groups. No mortality or overt morbidity was observed in the pups at any time (1–28 days), and growth curves did not differ among groups.

Morphologic Observations: DAAs

In newborn (1-day-old) pups, dissection of blood in the thoracic aorta occurred in 100% of pups born to dams treated with the highest 3 doses; at a dose of 6.125 mg/kg, the incidence of dissection was 91.7%. At the 2 lowest doses (0.096 and 1.530 mg/kg), only 2 aortic dissections were found (Table 1). The dissections were massive, greatly increased the diameter of the outer adventitia of the aorta, and frequently extended to the vessels of the aortic arch and superior to the carotid arteries (Fig. 1). The dissection of blood involved the outer third of the aortic media and was consistently located in the interstitial space between the media and the adventitia. The blood was limited by a markedly displaced (dilated) adventitia or surrounding structures, and thus formed a "false lumen." A low percentage showed complete tearing of the media (2/12 to 3/10 pups; Table 1, Fig. 2). No evidence of blood was seen in the pericardium (hemopericardium) or thoracic cavities (hemothorax).

Movat stain revealed that irregular areas of reduced, frayed elastic lamellae were found focally in vascular media of all vessels examined (Fig. 2). In some areas adjacent to the false lumen, we observed complete tearing of the media. In contrast, in other regions of the dissecting aneurysm within the same aorta, we observed considerable external dilation, with an intact medial lamina and narrowing of the original aortic lumen. In routine- and Movat-stained slides, there were no atherosclerotic lesions, intimal lesions of any kind, proximal aortic luminal stenosis, or inflammatory cellular infiltration illustrated in or around the dissection. The occurrence of focal, small areas of mural necrosis characterized by homogenous pink staining of vascular smooth muscle was observed in H-E-stained sections (Tables 1–3).

In 7- and 28-day-old pups, histologic studies revealed healing and healed vascular lesions consisting of localized collections of blood in vascular media (the false lumen) being organized by a fibrous reaction, and irregular areas of reduced, irregular elastic lamellae were found focally in the vascular media of all vessels examined (Tables 2 and 3, Figs. 2 and 3). On cross-section it was apparent that thrombus was present in the aneurysm and adventitia of the aortic wall encased by fibrous material. In 7- and 28-day-old pups, histologic studies revealed healing and healed vascular lesions consisting of localized collections of hemosiderin brown precipitation in the vascular media (the false lumen) and the fibrotic tissue surrounding the false lumen, which was verified by Prussian blue staining (Fig. 3). A fibrous reaction and irregular areas of reduced, frayed elastic lamellae were seen focally in vascular media of all vessels examined. No intimal tears were found in 7- or 28-day-old pups.

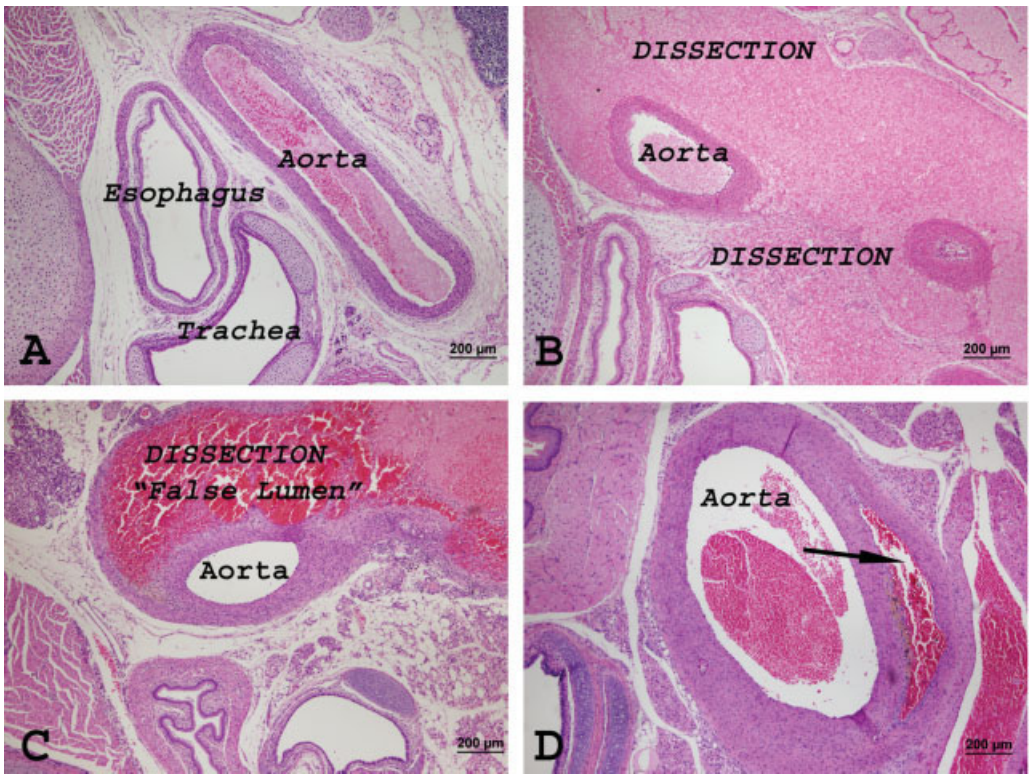

Figure 1. **A:** Thoracic section of a 1-day-old neonatal rat pup shows normal aorta and surrounding structures. **B:** In a 1-day-old pup born to a dam treated with semicarbazide (6.125 mg/kg/day), massive dissection of blood outside the aorta (DAA) is evident, and the original lumen of the aorta appears narrowed. **C:** A section from a 7-day-old rat pup shows remodeling of the aorta, with formation of a false lumen. **D:** At 28 days after birth DAA has remodeled and partially resolved (arrow). Note the maturation of structures, including thickening and enlarging of the aorta, maturation of the cartilage of the trachea, and so on. H-E stain; all photomicrographs were taken at the same power; scale bars = 200 μm.

In 7- and 28-day-old pups of dams exposed to 6.13 mg/kg (20% and 25%, respectively), extension of the dissection to the abdominal aorta was seen (Fig. 4). Pulmonary artery hemorrhage was noted at days 1 and 7 only (Tables 1 and 2). This lesion, which was rare except at the highest dose at day 7, consisted of adventitial hemorrhage without tears. When present, the hemorrhage extended along small pulmonary arteries throughout all lobes of the lungs.

Immunohistochemical Studies

Immunohistochemical staining with CD31 antibody demonstrated no new vascular endothelium in the false lumen of the DAA at all time points (Fig. 3A, C, and E). Immunostaining with SMC actin antibody illustrated that dissection generally took place between the vascular smooth muscle cells (VSMCs) of the outer third of the media, and in the adventitia of the 1-day-old pups. The healing process with developing collagen was associated with marked proliferation of positive anti-SMC actin staining VSMCs (Fig. 3B, D, and F).

Collagen and Elastin Quantification

No statistically significant differences were observed in total collagen or elastin content of dissected aortic specimens between control fetuses and those exposed in utero to semicarbazide at 6.125 mg/kg/day (Fig. 5).

Western Blot

No significant differences were observed in the expression of collagen type I, elastin, and fibrillin-1 in Western blot studies between dissected aortic specimens from control fetuses and those exposed in utero to semicarbazide at 6.125 mg/kg/day (Fig. 6).

DISCUSSION

DAA is a rapidly progressive tearing of the aortic media. It usually originates in the thoracic aorta and frequently results in sudden death, with few antecedent clinical symptoms in the days or months before the fatal event (Hagan et al., 2000, Januzzi et al., 2004). DAA occurs in younger adults, including teenagers (Helliker and Burton, 2003), and thus is distinguished from aneurysms of the abdominal aorta, which are characterized by male predominance and occurrence in older individuals (Sakalihasan et al., 2005). Furthermore, DAA and abdominal aortic aneurysms differ in typical pathologic characteristics. DAA is associated with splits or through-and-through tears through the vascular media that disrupt the vessel's integrity and cause rupture and extravasation of blood without

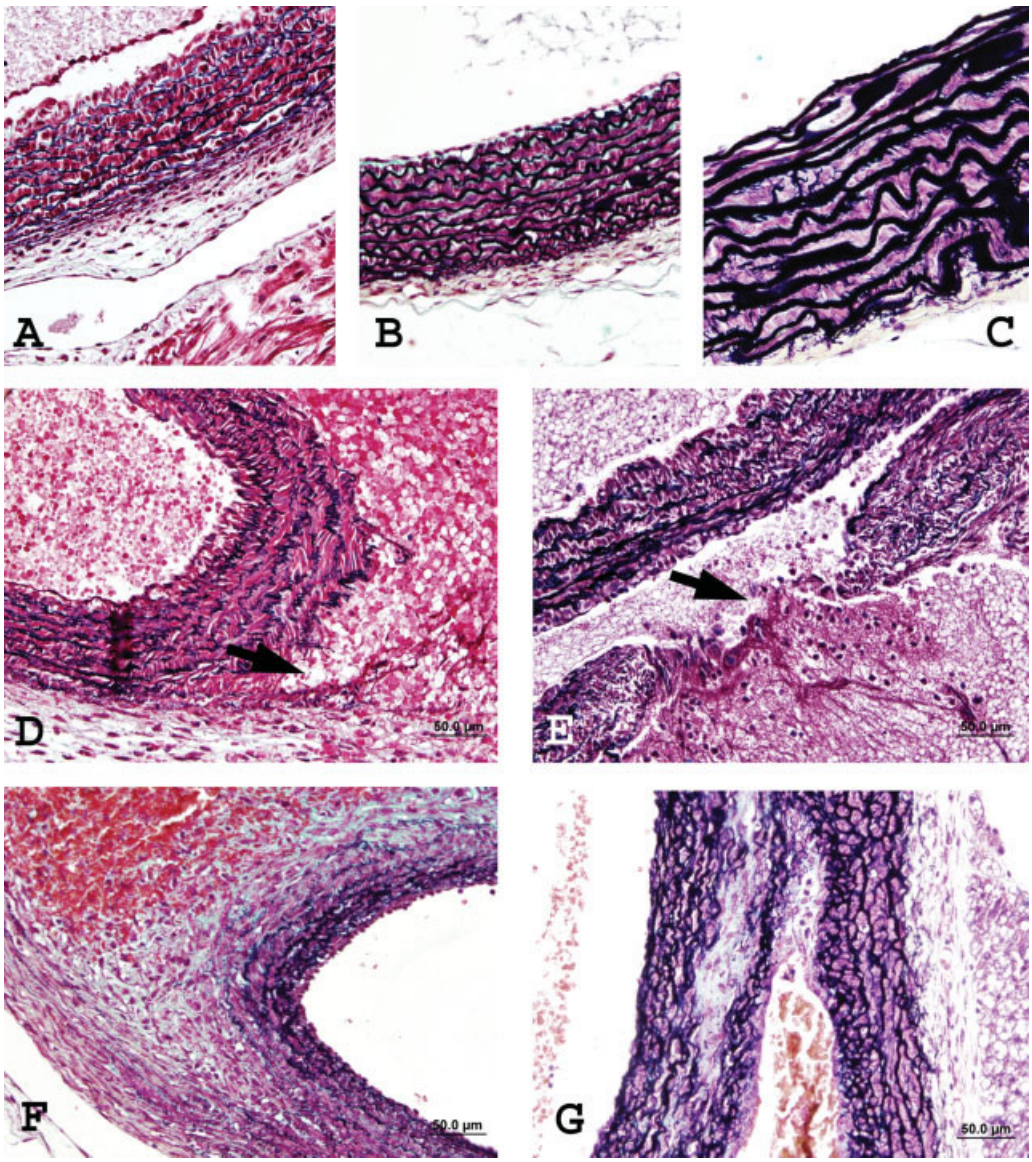

Figure 2. Higher-power views (all Movat-stained sections are shown at the same power) show normal, control aortic maturation at 1 (**A**), 7 (**B**), and 28 (**C**) days of age. Note alterations in the elastin laminae, with age, which stain black. **D:** In the aorta of a 1-day-old rat pup from a dam treated with semicarbazide (6.125 mg/kg/day), dissection occurs in the outer one-third of the media. Note the remnant of torn media and adventitia (arrow). Complete tears or disruption in media of the aorta (**E**, arrow) were also seen focally. **F:** Remodeling of dissected blood in 7-day-old rat pup consisted of fibrous tissue proliferation (blue) surrounding blood, or false lumen. **G:** At PPD 28 the remodeled false lumen contained residual blood, but elastic and fibrous tissue proliferation prominently surrounds the false lumen. All photomicrographs were taken at the same power; scale bars = 50 μm.

dilatation, whereas abdominal aortic aneurysms are characterized by marked dilatation, wall thinning, degenerative changes in the aortic media, and association with atherosclerosis. Indeed, the term "aneurysm" means "widening," which is characteristic of abdominal aneurysms but not of DAA.

DAA occurs relatively frequently in patients with Marfan syndrome, and in such cases it is believed that genetic defects in fibrillin lead to abnormal elastin formation as an underlying cause of dissection (Hayward and Brock, 1997; Pereira et al., 1997). The clinical condition of Ehlers-Danlos syndrome type IV, the vascular type, is also associated with dissection and rupture of aorta and other vessels (Pepin et al., 2000). In the majority of DAA cases, however, no etiology is evident.

Up to now, no useful, reproducible, nonlethal DAA models have been available for study (Ikonomidis et al., 2003). This is in contrast to the abdominal aortic aneurysm, which has been extensively modeled, predominantly through regional elastase infusion of the aorta, with consequent collagen and elastin degradation and accompanying increases in matrix metalloproteinase

Table 2
Thoracic/Abdominal Dissecting Aortic Aneurysm in 7-Day-Old Rat Pups from Dams Treated with Semicarbazide

Dose: mg/kg (n = pups/dams)	Thoracic aorta (%)	Branches of thoracic arch involved (%)	Abdominal aorta (%)	Necrosis in the vessel wall (%)	Pulmonary artery hemorrhage (%)
Control (4/2)	0	0	0	0	0
0.096 (6/2)	0	0	0	0	0
1.153 (6/2)	50	16.7	0	0	16.7
6.125 (8/3)	62.5	37.5[a]	25[a]	0	62.5

[a]1 dam's litter had no abnormal findings.

production (Boyle et al., 1998). The elastase-induced rat aortic aneurysm model is commonly used to investigate the pathogenesis and prevention of abdominal aortic aneurysm expansion (Anidjar et al., 1990). The utility of such models, however, is limited by a high mortality rate before aneurysms occur (Bigatel et al., 1999; Yamaguchi et al., 2000).

In the present study we have uncovered a remarkable developmental form of DAA. By treating dams with semicarbazide in the late gestational period (GDs 14–21 in the rat), one can universally induce a DAA at birth in rat pups. Hence, this developmental model is likely to be the result of disruption of late gestational or embryologic processes involved in vasculogenesis of the thoracic aorta.

The embryology of the aorta can be divided into 4 distinct periods of development in the rat (Nakamura, 1988). Until GD 12, no distinct vascular media are present, and the forming aortic arches are essentially primitive tubes lined by nascent endothelial cells (Hungerford and Little, 1999). At GDs 13 and 14, mesenchymal myoblasts migrate into the aortic wall, and the first ill-defined elastin aggregates are formed (Davis, 1995; Hungerford and Little, 1999). From GD 14 to 17, the layers of VSMC and elastic lamellae increase and become distinct, and an adventitia is recognizable by GD 17. From GDs 17 to 21 (term), extensive elastogenesis occurs, the matrix is elaborated, and the elastic lamellae are formed from the intima outward. VSMCs no longer proliferate extensively. Following birth, the elastic lamellae thicken and the matrix matures further (Gerrity et al., 1975; Gerrity and Cliff, 1975). Although the lamellar units are complete by week 5, the rheological and structural properties of the aorta reach near-maturity at 8 weeks of age (Katsuda et al., 2002).

In the present studies, DAA was universally present at birth in the absence of morphologic evidence of any other congenital structural defect in the outflow tract or great vessels. Specifically, there was no evidence of aortic or pulmonary artery anomalies, valvular defects, or outflow obstruction. Also, it is of interest that the dissection appears to originate in the ascending aorta (based on the high incidence of DAA and through-and-through tears or defects in that region). Thus, as is true of human DAA, dissection originates in the ascending aorta, and continuation or extension into carotids or distally in the aorta then occurs. This is consistent with known differences in the embryologic origins of the ascending aorta. VSMCs that migrate to the truncus arteriosus and eventually form the ascending aorta originate from a subpopulation of neural crest cells that differentiate to mesenchymal cells, the so-called "ectomesenchymal cells." The descending thoracic and abdominal aortas develop from paraxial rests of mesoderm lateral to the notochord. The dual origin of aortic VSMCs was first ascertained in avian species, but has since been confirmed in both the rat and mouse (Hungerford and Little, 1999). Hence, the cellular origin of the thoracic aorta is distinct from the tissue that eventually forms the abdominal aorta, supporting the concept that the pathobiology of aortic aneurysm formation in the thorax differs from that in the abdomen.

Although experimental models of aneurysm have focused on the abdominal location, few experiments have attempted to mimic thoracic aneurysm, or DAA. Transplantation of thoracic aortas from one adult guinea pig to another was found to result in vascular rejection accompanied by marked inflammation, dilatation, and rupture of the transplanted vessel (Allaire et al., 1994). Ikonomidis et al. (2003) produced dilated thoracic aortic aneurysms by applying topical calcium chloride directly to the vessels' external surface. The method reproducibly caused dilatation and medial thinning accompanied by breakdown of adventitial collagen, but dissection of media was not demonstrated. Dissection is not a characteristic of these previous models of thoracic aortic aneurysm.

Table 3
Thoracic/Abdominal Dissecting Aortic Aneurysm in 28-Day-Old Rat Pups from Dams Treated with Semicarbazide

Dose: mg/kg (n = pups/dams)	Thoracic aorta (%)	Branches of thoracic arch involved (%)	Abdominal aorta (%)	Necrosis in the vessel wall (%)	Pulmonary artery hemorrhage (%)
Control (6/2)	0	0	0	0	0
0.096 (10/2)	0	0	0	0	0
1.153 (12/2)	16.7	0	8.3	0	0
6.125 (10/3)	100 [20][a,b]	60 [40][a,c]	20 [10][a,c]	0	0

[a]Hemosiderin present, incidence shown in brackets.
[b]1 dam's litter had no findings.
[c]2 dams' litters had no findings.

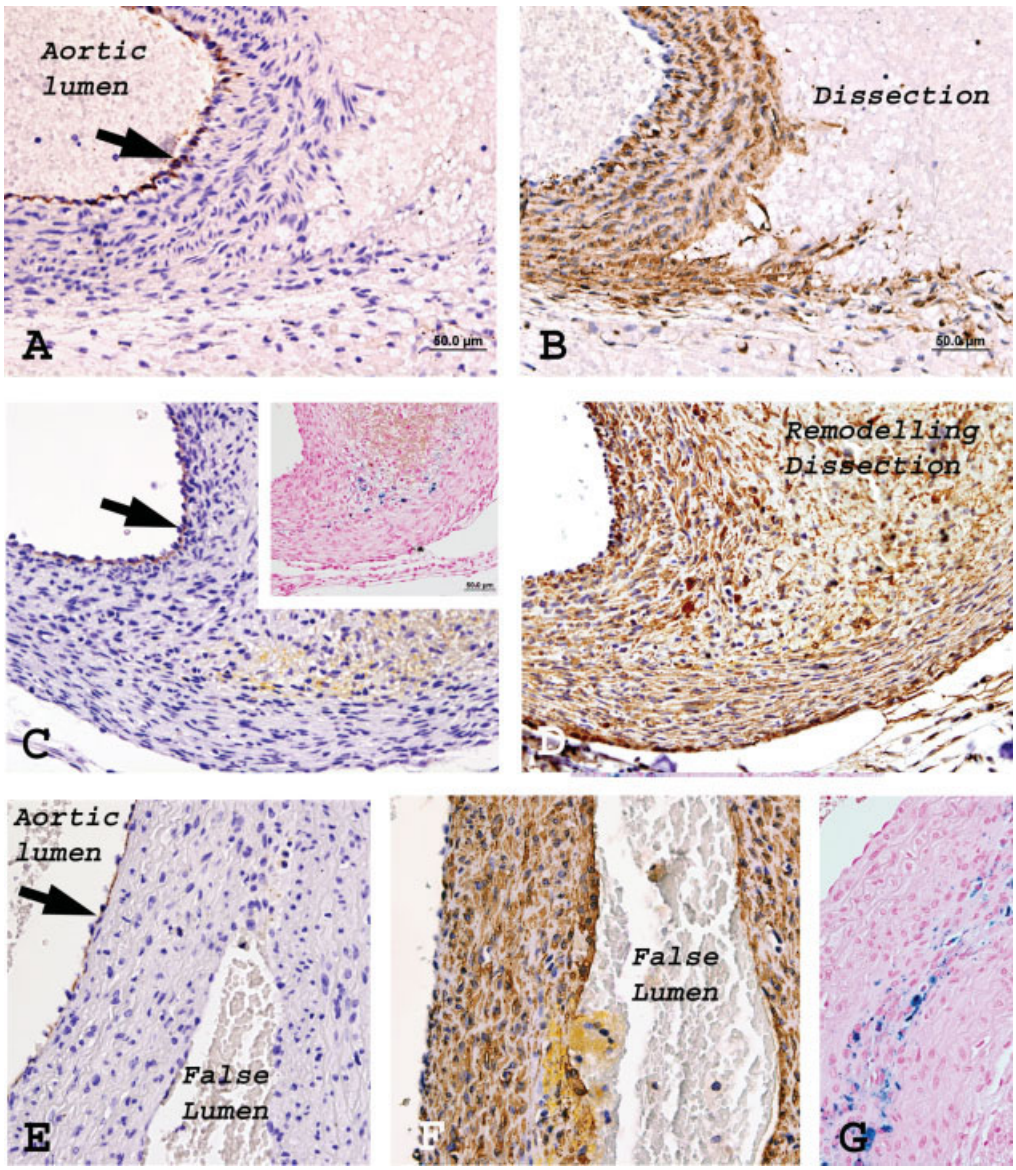

Figure 3. Immunohistochemical study. Staining for CD31 (for endothelial cells) is shown in the left 3 panels (**A**, **C**, and **E**). Staining for α-SMC actin is shown in the right 3 panels (**B**, **D**, and **F**). Endothelial cells line the aorta homogenously (arrow). Note the remodeling of dissection in 1-day-old (A), 7-day-old (C), and 28-day-old (E) pups. Endothelial cells do not line the false lumen and are not found during remodeling of dissection. B: Vascular smooth muscle cells (VSMCs) are split by dissection in the outer one-third of the media in a 1-day-old rat pup. In inset in C, iron stain shows deposits indicative of resolving blood (hemosiderin) in remodeling DAA in 7-day-old pup. D: Remodeling of dissection in a 7-day-old pup shows proliferation of VSMCs into dissected blood. F: In a 28-day-old pup, aortic dissection is remodeled by VSMCs that surround the false lumen. **G:** The organizing false lumen contains iron stained by Prussian blue stain, indicative of hemosiderin. All photomicrographs were taken at the same power; scale bars in A–C (inset) are 50 μm.

The present developmental studies were inspired by previous studies in weanling rats (21–42 days old) (Langford et al., 1999) and in in vitro vascular cells (Langford et al., 2002). In those earlier studies, specific inhibition of SSAO (a vascular enzyme with the highest activity in the aorta) resulted in striking pathologic changes that bore many similarities to a dilated type of aortic aneurysm. Specifically, the previous lesions showed marked disorganization of elastin fibers, increased vascular circumference, and aberrations in connective tissue metabolism, including decreases in production of mature elastin concomitant with increased collagen.

However, the results of the present characterization of this new, reproducible, nonlethal model of DAA diverge from our earlier vascular findings in weanling animals treated with semicarbazide. The striking lesions of DAA in this study showed little or minor alterations in elastin or collagen morphology in the aorta, whereas in weanling animals the aortic elastin was markedly abnormal (with irregular, discontinuous, and fragmented fibers). Also, ar-

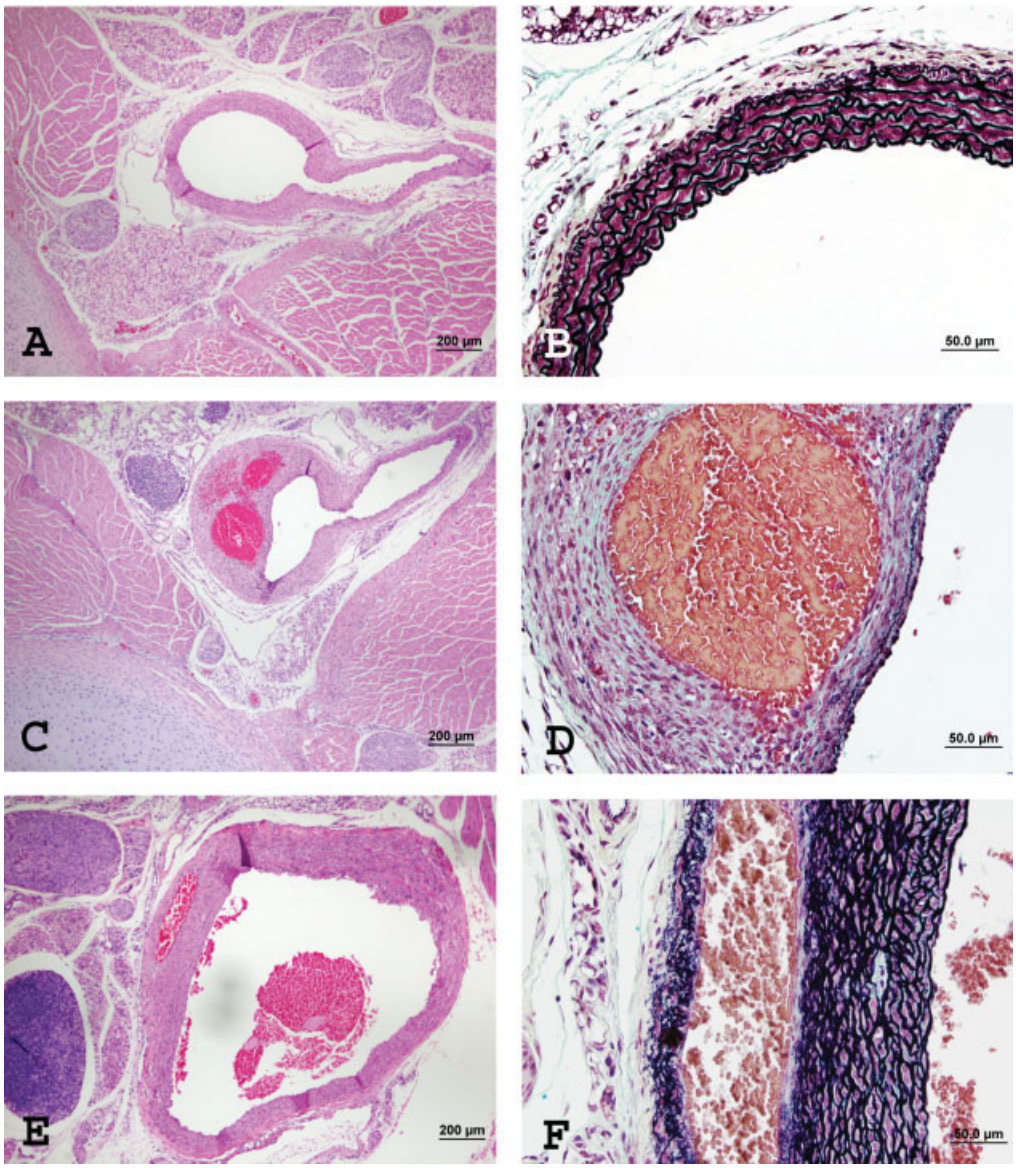

Figure 4. Extension of DAA to abdominal aorta. Left panels: H&E stained sections. Right panels: Movat-stained sections. A control abdominal aorta (**A:** low power; **B:** high power) at the level of the renal artery is shown. **C:** A 7-day-old rat pup with extension of DAA to abdominal aorta with false lumen. **D:** Remodeling of DAA in 7-day-old rat pup shows predominance of fibrous tissue. Remodeling in a 28-day-old rat pup (**E**) with a small false lumen remaining is surrounded by irregular, discontinuous elastic tissue (**F**).

eas of increased collagen deposition were noted in the media of weanling experimental rats.

Similarly, the biochemical changes of diminished mature elastin and increased collagen found in weanling animals in the previous experiments were not evident in the present study. Indeed, no overt changes in collagen were found biochemically or by Western analysis. Nevertheless, it seems likely that the remarkable and virtually universal aortic lesions of DAA are due to some defect of structural molecules, such as elastin or collagen. Because it is well known that the virtually identical lesions of DAA found in some patients with Marfan syndrome are related to genetic defects of fibrillin-1 (Hayward and Brock, 1997), we also examined the possible role of this molecule, which is essential for the development of normal elastin lamellae (Brooke et al., 2003). Our results, however, demonstrated no overt defect in fibrillin-1 protein by Western blot analysis of the aortas of term fetuses from treated dams.

The morphologic similarities to the DAA seen in Marfan syndrome argue strongly that some defect in the structure of elastin probably underlies the striking shearing seen in the present experiments. Specifically, the DAA in our model appears to begin in the arch of the aorta (likely in the ascending portion, based on the prevalence of lesions in that location and the finding of through-and-through tears of media there). Similarly to DAA in humans, the dissection apparently continues inferiorly and superiorly down the abdominal aorta and into the carotids. The re-

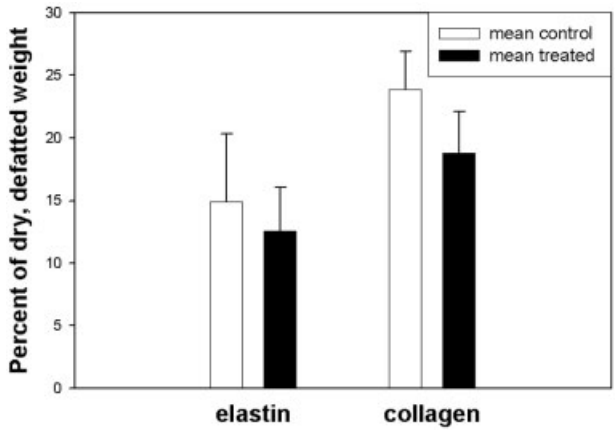

Figure 5. Dry, defatted aortic elastin and collagen fractions harvested from pooled fetuses (killed at GD 20) from semicarbazide-treated dams. Each bar set represents the mean percentage ±SD of dry, defatted weight of elastin (left) and collagen (right). The control group (white bars) consisted of 39 fetuses from 3 control pregnant rats, and the treated group (black bars) contained 59 fetuses from 5 treated pregnant rats.

modeling that is demonstrated in our time-course study (as long as 28 days) is also similar to the resolution or healing that is frequently seen in the DAAs of surviving patients (P.J.B., personal observation).

Further support for the concept that altered collagen or elastin metabolism is the mechanism of DAA formation in our model is found in several genetic studies that reported somewhat similar lesions. In mice in which the lysyl oxidase gene was targeted, homozygous null mice died shortly after delivery, demonstrated tortuous aortas, and were presumed to have died of diaphragmatic rupture (Hornstra et al., 2003). In mice with a deletion in the first intron of the *Colla1* gene, dissecting abdominal aortic aneurysms to the level of the renal arteries were documented for up to 18 months, although the exact site of the dissection and rupture was not specified (Rahkonen et al., 2004). Hemopericardium, hemothorax, or hemomediastinum was noted at necropsy in homozygous mutant fibrillin-1 mice with an age range of <1 week to 12 months, suggesting a vascular etiology (Pereira et al., 1997). Similarly, a null mutation in the *Col3a1* gene in type III collagen caused a high lethality from abdominal aortic dissecting aneurysms and resulted in defective wound healing. In these studies, only 10% of the homozygous mutant animals survived to adulthood (Liu et al., 1997). These previous studies suggest that the basis of our developmental model is interference by semicarbazide with collagen or elastin metabolism during the late gestational period.

In summary, we have described a reproducible, nonlethal model of DAA in newborn rats from dams treated with the SSAO inhibitor semicarbazide on days 14–21 of in utero development. The model has no discernible mortality or morbidity (in the 28-day period of observation) and provides investigators a model in which they can examine the potential long-term effects of vascular remodeling of DAA. Although our initial data do not reveal a clear mechanism for DAA with regard to collagen, elastin, or other matrix defects, our working hypothesis is that DAA is caused by aberrations of elastogenesis or matrix formation during the critical embryologic period of vasculogenesis. These studies also serve to raise awareness about the possible deleterious vascular effects that in utero exposure to environmental chemicals may cause during the latter third of fetal development.

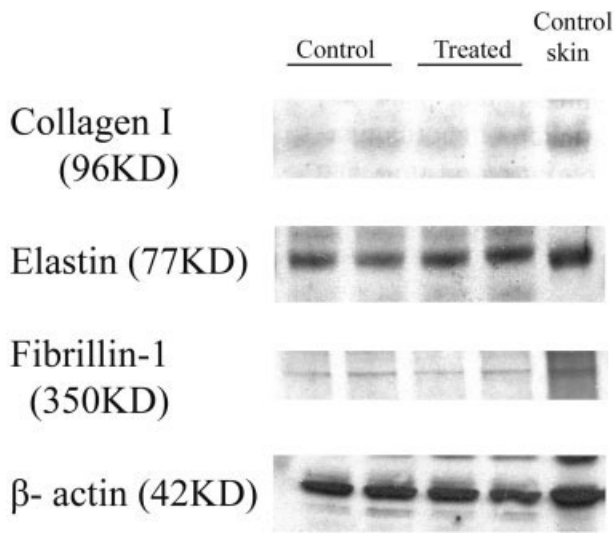

Figure 6. Representative Western blot analysis for expression of collagen type I (96 kDa), elastin (77 kDa), fibrillin-1 (350 kDa), and β-actin (42 kDa). Pooled fetal aortic samples from 2 control (11 fetuses pooled per pregnant rat) and 2 treated pregnant rats (10 fetuses pooled per pregnant rat) were loaded (details in text). An homogenized sample of skin from the control animal was loaded as a positive control for extracellular matrix proteins.

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