Novel experimental model of enlarging abdominal aortic aneurysm in rabbits

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Objective: **This study tested the hypothesis that an experimental model of abdominal aortic aneurysm in rabbits results in progressive enlargement when induced by a combination of periaortic elastase administration and aortic coarctation.**

Methods: **Male New Zealand white rabbits were randomly divided into four groups: (A) stenosis (n = 12), (B) elastase (n = 12), (C) aneurysm (n = 15), and (D) control (n = 12). The stenosis group received an extrinsic coarctation below the right renal artery, the elastase group received a 10-minute administration of 60 μL elastase (1 U/μL) in a 1.5-cm aortic segment, the aneurysm group received stenosis and elastase, and a sham operation was performed in the control group. The aortic diameter was measured after 1, 2, 4, 8, and 16 weeks, and animals were subsequently euthanized for histopathologic and immunohistochemical studies.**

Results: **All animals in the aneurysm group developed aneurysm by 2 weeks after treatment, with average diameters of 5.21 ± 0.74 mm by 2 weeks, 6.23 ± 1.10 mm by 4 weeks, 7.87 ± 0.50 mm by 8 weeks, and 9.40 ± 0.36 mm by 16 weeks. Aortic diameter dilated progressively, and all aneurysms developed by 4 weeks in the stenosis group (4.17 ± 0.22 mm). Only one aneurysm was seen in the elastase group by week 1 (3.60 ± 0.64 mm), and no aneurysm formed in the control group by week 8 (2.47 ± 0.38 mm). The aneurysm group exhibited less media thickness, elastin content, and endothelial recovery, but stronger expression of matrix metalloproteinase 2 and 9 and rabbit macrophage compared with the control group.**

Conclusions: **This novel rabbit abdominal aortic aneurysm model with a gradually enlarging diameter is simply and reliably induced, appropriately mimicking human aortic aneurysm disease. (J Vasc Surg 2015;62:1054-63.)**

Clinical Relevance: **Experimental abdominal aortic aneurysm (AAA) models can be useful to investigate its pathogenesis and to mimic human AAA disease. Medial injury aneurysms induced by elastase or calcium chloride are popular models. Unfortunately, the elastase-induced aneurysm dilates <70% and heals spontaneously. Periaortic calcium chloride application does not always induce reliable AAA formation. In this study, a novel AAA model was induced in rabbits by periaortic elastase incubation and aortic coarctation. Elastase incubation and turbulent flow caused by coarctation could work in concert to develop AAA progressively to overcome the self-healing phenomenon and to better mimic human AAA disease.**

Abdominal aortic aneurysm (AAA) is a life-threatening disease,[1] but the pathogenesis of AAA remains poorly understood.[2] Experimental models can be useful to investigate pathogenesis and to mimic human AAA disease for translational research. Small animal aortic aneurysm models have been characterized by (1) genetic predisposition, (2) medial injury, and (3) hemodynamic perturbation.[3] In particular, medial injury AAA models induced by elastase or calcium chloride are popular.[4-13] Aneurysm dilation after elastase treatment was <70%.[5,7,8] Further, periarterial elastase-induced AAA does not progress and heals spontaneously.[7] Elastase injury does not reliably result in aneurysm formation unless the adventitia is removed.[14,15] In addition, periaortic calcium chloride application does not always induce AAA formation, even when an arbitrary threshold of 20% diameter increase is used to define AAA.[16]

Human aortic aneurysms are characterized by progressive expansion and eventual rupture.[17] Medial injury aneurysm models, such as elastase-induced and calcium chloride-induced AAAs, cannot cause continuous enlargement and eventual rupture.[13] However, Mata et al[18] combined traumatic injury to the external aortic wall and extrinsic stenosis to create a rat AAA model with a dilation ratio of >300%. This resulted in progressive dilation over 15 days, indicating that hemodynamic changes caused by stenosis play an important role in poststenotic dilatation

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(PSD), a fusiform dilation induced by stenosis or coarctation.

The purpose of this study was to investigate whether the periaortic administration of elastase and aortic coarctation could work in concert to overcome the self-healing phenomenon and to better mimic human AAA disease by creating progressive AAA.

METHODS

This study was conducted under the approval of the Animal Experimental Ethics Committee of the China Medical University.

Animals and experimental groups. Fifty-one male New Zealand white rabbits were randomly divided into four groups: (A) stenosis (group S, n = 12), (B) elastase (group E, n = 12), (C) aneurysm (group SE, n =15) and (D) control (sham, n = 12). The aorta was narrowed with an external suture below the right renal artery in group S. The aorta was bathed in elastase solution (60 μL, 1 U/μL) for 10 minutes in the group E. Group SE, the aneurysm group, received stenosis and elastase, and a sham operation was performed in the control group.

A novel AAA model. Rabbits were anesthetized with intravenous injection of sodium pentobarbital (30 mg/kg), and a laparotomy was performed under sterile conditions. The mid infrarenal aorta was carefully isolated from the vena cava and surrounding tissues. A 1.5-cm aortic segment was circumferentially wrapped with a piece of sterile gauze, and the adventitia of this segment was then incubated with 60 μL porcine pancreatic elastase (1 U/μL, ≥30 U/mg; pI 9.5, pH 8.1-8.9; Shanghai Kayon Biological Technology Co, Ltd, Shanghai, China) for 10 minutes in the group E. The elastase diffuses into the aortic wall, and destroys the whole wall.[9,12] After the conclusion of the topical application of elastase, the gauze was removed and the treated segment was irrigated twice with saline.

In group SE, the aneurysm group, elastase was administered as above, and a ligature of cotton thread was performed just above the treated segment. The segment was constricted with a pipette tip, and then the tip was immediately removed, reducing the vessel lumen to the diameter of the tip. Group S received an extrinsic stenosis and periaortic administration of saline instead of elastase. A sham operation was performed in the control group. The abdomen was closed with continuous running suture, and the animal was allowed to recover in a warming cage.

Intravenous digital subtraction angiography and transabdominal ultrasound imaging. All rabbits underwent serial intravenous digital subtraction angiography imaging after 1, 2, 4, 8 and 16 weeks, as previously reported.[9,12,13,19] A 22-gauge angiocatheter was placed in the ear vein, and iodinated contrast material (6 mL) was injected by hand into the ear vein ≤3 seconds. Aortic diameter measurement was performed with reference to a 1-cm-length external scale by a person blinded to treatment. Serial transabdominal ultrasound imaging was performed to study the hemodynamic changes. Wall shear stress (WSS) was calculated using Poiseuille's formula, as previously reported.[20] $WSS = 4\mu Q/\pi r^3$, where Q is the blood flow rate (mL/s), μ is the blood viscosity (0.035 poise), and r is the arterial lumen radius (cm). WSS was calculated where the radius of the treated segment was largest. Successful AAA formation was defined as the treated aorta enlarged at least 50% compared with the normal diameter.

Animal euthanasia and histopathology. In three rabbits in each group, a second laparotomy was performed after the ultrasound study. Animals were anesthetized as above and pressure perfusion-fixed with 10% buffered paraformaldehyde solution. Aortic tissues were embedded in paraffin and cut into 5-μm sections. The slides were stained with hematoxylin and eosin, elastic van Gieson dye for elastin, and picrosirius red stain in conjunction with polarized light microscopy for identifying type I collagen (orange-red fibers) and type III collagen (yellow or green fibers). Images of the sections were analyzed by using Image-Pro Plus 6.0 software (Media Cybernetics Inc, Rockville, Md), as previously described.[21] In the hematoxylin and eosin-stained sections, the media thickness, intima thickness, and intima-media thickness (IMT) were measured as the average thickness of six fields of cross-sections. Semiquantitative analyses for elastin and collagen content were calculated by measuring the elastin and collagen area under fields at original magnification ×200.

Immunohistochemical analysis. Samples were analyzed for matrix metalloproteinase 2 (MMP2) and MMP9, endothelial cell marker (CD31), and rabbit macrophage (RAM11). The UltraSensitive streptavidin-peroxidase kit (KIT-9701, Maixin Bio, Fuzhou, China) was used as previously reported.[9,12,13] Tissue sections were incubated with 1% H_2O_2 in methanol for 20 minutes to block endogenous peroxidase activity. Nonspecific binding was blocked with 10% goat serum for 30 minutes at room temperature, then antibodies to anti-MMP2 antibody (4D3, ab2462; 1:200 dilution; Abcam, Hong Kong, China), anti-MMP9 antibody (56-2A4, ab58803; 1:300 dilution; Abcam), monoclonal mouse antirabbit macrophage (1:100 dilution, clone RAM11, code M0633; Dako, Glostrup, Denmark), and monoclonal mouse antihuman CD31 (Ready-to-Use, Clone JC70 A, Code IR610; Dako) were incubated overnight in a humid chamber at 4°C. After three washes in phosphate-buffered saline, sections were incubated with biotinylated antimouse secondary antibody for 20 minutes, followed by the streptavidin-peroxidase method, according to the manufacture's protocol. Diaminobenzidine tetrahydrochloride was used to visualize the sections. All slides were examined at original magnification ×200 and scored. Any slides that exhibited strong positive immunostaining expression (>50%) were scored as 3, moderate positive expression (10%-50%) was scored as 2, and weak expression (<10%) as was scored as 1. Endothelial recovery was calculated by the length of the CD31-positive layer divided by the length of the internal elastic lamina.

Immunofluorescent analysis of smooth muscle cells. After endogenous peroxidases were blocked, sections were incubated with mouse monoclonal anti-α-smooth