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Tissue expander stimulated lengthening of arteries (TESLA) induces early endothelial cell proliferation in a novel rodent model☆

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ABSTRACT

Background: We examine the mechanism of aortic lengthening in a novel rodent model of tissue expander stimulated lengthening of arteries (TESLA).
Methods: A rat model of TESLA was examined with a single stretch stimulus applied at the time of tissue expander insertion with evaluation of the aorta at 2, 4 and 7 day time points. Measurements as well as histology and proliferation assays were performed and compared to sham controls.
Results: The aortic length was increased at all time points without histologic signs of tissue injury. Nuclear density remained unchanged despite the increase in length suggesting cellular hyperplasia. Cellular proliferation was confirmed in endothelial cell layer by Ki-67 stain.
Conclusions: Aortic lengthening may be achieved using TESLA. The increase in aortic length can be achieved without tissue injury and results at least partially from cellular hyperplasia. Further studies are required to define the mechanisms involved in the growth of arteries under increased longitudinal stress.

Midaortic syndrome (MAS) is a rare but serious cause of hypertension in children and young adults. It is characterized by coarctation of a portion of the abdominal aorta with variable involvement of the renal and mesenteric arteries. When left untreated, most patients die of complications of hypertension by the fourth decade of life [1]. Anatomic correction via bypass grafting, aortoplasty or angioplasty and stenting are the current standard management strategies employed for symptomatic patients. However, in young patients who have not yet completed growth, the use of prosthetic grafts for reconstruction often results in the need for additional procedures later in life for revision or replacement of the graft as the child grows [2]. Therefore, development of a procedure that utilizes only native tissue at the time of the original repair would be ideal. Our group recently reported the use of a tissue expander to induce lengthening of the normal distal aorta in a young child with MAS, with successful resection of the diseased segment and primary end-to-end anastomosis [3]. In the plastic surgery literature, tissue expanders have been used in animal models and humans to acutely elongate peripheral arteries for coverage of arterial defects, but the technique has not been applied to larger, central vessels, and data regarding the morphologic effects on vessels in vivo are limited [4–7]. Using a novel rodent model, we aimed to investigate the morphologic and cellular response of the aorta to TESLA.

1. Materials and methods

1.1. Animal model

The animal work was performed within the guidelines of the Boston Children's Hospital Institutional Animal Care and Use Committee (IACUC) approved protocol 11-09-1909. Adult male Sprague–Dawley rats (350–450 g) were anesthetized and the abdominal aorta was exposed via a midline incision and medial visceral rotation. The caudal mesenteric vessels were identified bilaterally, ligated and divided. The aortic bifurcation was identified and an adventitial marking suture was placed at the bifurcation and approximately 2 cm superior to the bifurcation. The distance between marking sutures was recorded as the initial length measurement. The aorta and IVC were then separated from surrounding tissues, with ligation of posterior perforating vessels performed when needed to free up the vessels for the entire length between marking sutures. In experimental animals ($n = 16$), a 2×4 cm rectangular expander (PMT Corporation item# 3611-322) was placed

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in the retroaortic space and secured to the psoas muscles (Fig. 1). The accompanying port was tunneled through the abdominal wall and secured subcutaneously in the animal's right flank. The port was then injected with 4 mL of sterile saline. In sham animals (n = 10), an equivalent strip of expander material was placed in the retroaortic space and secured to the psoas muscles. The animals were all dosed with 5 mL of subcutaneous crystalloid fluid resuscitation at the conclusion of the case. The rats were allowed to recover from anesthesia and were returned to single cages for 2–7 days. Meloxicam was administered for pain control daily for 3 days. After 2, 4, or 7 days, animals were euthanized and the aorta and IVC were clamped above the aortic marking suture and below the bifurcation. The vessel bundle was then excised and the distance between the marking sutures was recorded with the vessel hanging vertically with a 7 g weight secured to one end to counteract vessel elasticity and to allow for fair comparisons in length between specimens. The tissue was then flushed with heparinized saline and fixed in 10% formalin.

1.2. Length analysis

Following placement of the adventitial marking sutures in the initial surgery, the baseline distance between the sutures was measured in vivo without any applied tension. Final length was obtained at 2, 4 or 7 days by measuring the distance between sutures after the aorta had been removed from the animal. Measurements were performed at the time of sacrifice by two unblinded investigators. These measurements were used to calculate the percent change in length from baseline. Two-way analysis of variance (ANOVA) was used to compare groups and changes over the time points.

1.3. Histology

5–7 μm transverse sections were stained with hematoxylin and eosin and photographed using light microscopy at 40× magnification. Total thickness of the aortic wall (media and intima) was measured at four equally spaced sites around the circumference of each section using Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, CA) by two observers. Average wall thickness for each specimen was determined and data were analyzed using two-way ANOVA to compare groups and time points with a repeated measures factor to account for results from multiple observers. F-tests were used to assess overall significance with post-hoc Fisher least significance comparisons for detecting specific group and time point effects.

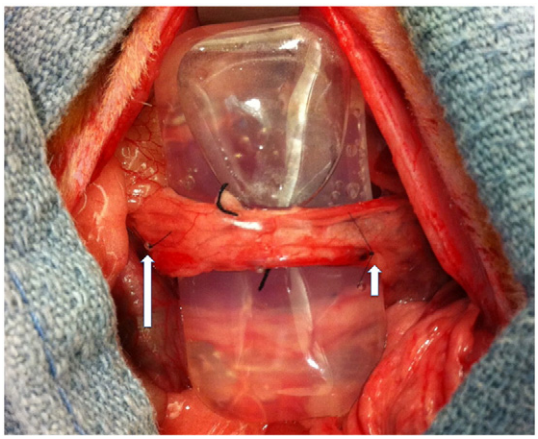

Fig. 1. Photograph of tissue expander in position and inflated posterior to aorta and inferior vena cava. Inferior marking suture (long white arrow) and superior marking suture (small white arrow) are approximately 2 cm apart.

1.4. Immunofluorescence

Formalin fixed paraffin embedded (FFPE) sections of 5–7 μm were dewaxed and rehydrated in a series of graded alcohols. Antigen retrieval was performed with Antigen Unmasking Solution (1:100 dilution, Vector Laboratories, Burlingame, CA, Cat# H-3300) at 98°F for 30 minutes. After cooling to room temperature, sections were washed in 1× PBS and blocked for 20 minutes using 4% goat serum, 0.1% Triton in PBS. Sections were incubated overnight with primary antibody followed by three washes in PBS and labeling with secondary antibody. Sections were then mounted in VECTASHIELD Mounting Medium with DAPI (Vector Laboratories, Burlingame, CA, Cat# H-1200). Antibodies utilized included Ki-67 monoclonal antibody (1:50 dilution, Thermo Scientific, Waltham, MA, Cat# RM-9106-S0), von Willebrand Factor (1:500 dilution, Abcam, Cat#ab778), and Alexa Fluor Dyes (1:500 dilution, Jackson Immune Research Lab, West Grove, PA, Cat# 111-546-003, 111-586-003).

Each section was photographed using a Nikon Eclipse fluorescent microscope at 200× magnification. The area including aortic wall (media and intima) was isolated from images and surface area was measured with Adobe Photoshop CS3 (Adobe Systems Incorporated, San Jose, CA). Nuclei staining positive for Ki-67 were manually counted. These counts were repeated by a blinded second observer. Nuclei staining positive for DAPI were counted using Image-Pro Analyzer Version 6.3 (Media Cybernetics, Inc., Rockville, MD). Nuclear density (DAPI count/surface area) was calculated using surface area of the isolated wall and the previously generated DAPI count from each section.

Proliferation index (Ki-67 count/DAPI count × 100%) was calculated for each image by averaging both manual counts performed for Ki-67 in each section and dividing by the computer generated DAPI count. Data were analyzed using two-way factorial ANOVA, accounting for multiple observers and repeated measures, to compare between groups and over time points.

Correlation between observers was very high with Pearson r values over 0.95, which provided a good rationale for combining measurements from the two observers. Statistical analysis was performed using IBM SPSS Statistics (version 21.0, IBM, Armonk, NY). Two-tailed values of $p < 0.05$ were considered statistically significant.

2. Results

2.1. Length

The percentage change in length was significantly increased in experimental animals at two (16 ± 6.2, $p = 0.001$) four (13.5 ± 9.5, $p = 0.026$) and seven (18.5 ± 6.7 $p = 0.009$) day time points compared to sham animals at comparable time points (Fig. 2A). Interestingly, the sham lengths appeared to be decreased in the early time points compared to baseline measurements at the time of initial surgery, although this change was not statistically significant. In addition, the majority of the increased length in the experimental animals was already present in the 2 day group and the additional increase in the length by day 7 was not significant.

2.2. Muscular wall thickness

Average wall thickness was decreased in experimental 2-day animals when compared with sham animals at 2 days (0.103 mm vs. 0.122 mm; $p = 0.04$). No significant differences were found in wall thickness between experimental, sham, or normal control groups at four or seven day time points (Fig. 2B). There also did not appear to be a change in wall thickness over the duration of the experiment among sham animals or experimental animals. All samples appeared grossly normal on H&E staining, with intact endothelium, muscular layer and elastic lamina (Fig. 3).