Topical VEGF Enhances Healing of Thoracic Aortic Anastomosis for Coarctation in a Rabbit Model

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Background—Recurrent stenosis after extended end-to-end anastomosis for aortic coarctation is the primary indication for further interventions in children. Tension because of the extended resection and local arterial wall hypoxia are possible pathogenetic mechanisms. We hypothesized that (1) tension interferes with healing and (2) that vascular endothelial growth factor (VEGF), a hypoxia sensitive angiogenic inducer, may enhance healing of the vascular anastomosis.

Methods and Results—In a model of coarctation repair, rabbits underwent thoracic aortic end-to-end anastomosis after transection (no-tension; n=15), resection of an aortic ring (tension; n=14) or resection and topical VEGF treatment (0.75 μg VEGF165; tension+VEGF; n=14). Gross and histologic characteristics of the aortic wall were assessed at 1 week, 1 and 2 months. In the tension only group at 1 month, the severity of vascular remodeling was increased with fibrosis and calcification compared with controls. At 2 months, this group also revealed more luminal stenosis (29% versus 19%; P<0.001). Exogenous VEGF resulted in significantly less fibrosis, calcification and chondroid metaplasia at 1 month (P<0.05) and luminal area was only reduced 3% at 2 months (P<0.001 versus tension group).

Conclusions—In a rabbit model of coarctation repair, the addition of tension on the vascular anastomosis resulted in poor healing and luminal stenosis. Topical VEGF maintained luminal integrity by decreasing fibrosis and calcification. These findings suggest that topical VEGF may be a promising new strategy to enhance healing and improve the outcome of vascular anastomoses for coarctation of the aorta. (Circulation. 2003;108[suppl II]:II-150-II-154.)

Key Words: coarctation • restenosis • growth factors • remodeling • aorta

Coarctation of the aorta is often associated with aortic arch hypoplasia,1-3 and repair by resection with extended end-to-end anastomosis is currently the procedure of choice at most centers.1,2,4,5 However, recurrent stenosis can occur leading to further invasive procedures.1,2,5,6 The surgical procedure, itself, may contribute to this complication. It requires resection of all ductal tissue, and in case of arch hypoplasia, greater extension of the anastomosis, thus increasing the distance between the severed aortic ends. This can lead to tension at the site of anastomosis. Moreover, rupture of the adventitial vasa vasorum by transection of the aorta causes prolonged local hypoxia at the anastomotic site7,8 as hypoxia of the injured arterial wall has been demonstrated in an experimental model even at 2 weeks after an arterial end-to-end anastomosis.9

The hypoxia-regulated glycoprotein vascular endothelial growth factor (VEGF)10 is a potent angiogenic factor that acts through 2 receptors, Flt-1(VEGFR-1) and Flk-1(VEGFR-2).11 In addition to the direct angiogenic effects, VEGF is capable of inducing endothelial cell survival12,13 and is expressed in normal and atherosclerotic human arteries14 suggesting a role in the maintenance and repair of the vascular wall. VEGF stimulates endothelial production of nitric oxide and prostacyclin, 2 mediators predicted to have a vascular protective effect.12 We previously reported the beneficial effect of topical VEGF on increasing neovascularization and improving healing of an avascularized tracheal autograft in an experimental model15 supporting a role for VEGF in the setting of surgically related tissue ischemia.16

To examine the impact of tension on an arterial end-to-end anastomosis we used a rabbit model of descending thoracic aortic end-to-end anastomosis, in which tension is created by resection of an aortic ring. We hypothesized that tension increases vascular remodeling, interferes with healing of the arterial end-to-end anastomosis and contributes to vascular stenosis. Moreover, surgical disruption of the vasa vasorum induces local hypoxia, thus, topicaly delivered pro-angiogenic VEGF may enhance the healing of an arterial end-to-end anastomosis and diminish vascular stenosis.

Methods

Animals
Forty-five male New Zealand White rabbits, 9 weeks of age (sexually immature) and with a mean weight of 2.47±0.17 kg (2.2
kg to 2.8 kg), were maintained under standardized conditions with free access to water and standard rabbit diets (Hi-Fiber 5P25, Prolab). All experiments were performed with the approval of the Animal Care and Use Committee of Children’s Memorial Institute of Education and Research. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH publication No. 85 to 23, revised 1985).

**Surgical Procedure and Treatment**

The animals were anesthetized by subcutaneous injection of acepromazine (0.5 mg/kg) followed by intramuscular injection of xylazine (7 mg/kg) and ketamine (40 mg/kg). After intubation using a sterile 3F endotracheal tube anesthesia was maintained with inhaled isoflurane (1% to 2%). All surgical procedures have been performed by the same trained cardiac surgeon (R.G.S.) using identical technique and vascular clamps. Under sterile conditions a left thoracotomy was performed and the descending thoracic aorta was dissected free just below the left subclavian artery. If necessary, intercostal arteries were divided. After proximal and distal aortic cross-clamping, the thoracic aorta was transected or a 10-mm ring of aorta was resected and the vehicle PBS/0.1% albumin or rhVEGF165 was delivered intraluminally. An end-to-end anastomosis using 7-0 running polypropylene (Ethicon Inc, Sommerville, NJ) suture technique was performed. Before finishing the anastomosis the severed ends were flushed with heparinized saline to prevent thrombus formation. Once hemostasis was achieved the vehicle only or VEGF was administered to the adventitial surface. After an exposure time of 10 minutes, the chest was closed with 2 layers of continuous 3-0 Vicryl sutures (Ethicon, Inc) for the muscular layer and continuous 3-0 Vicryl sutures for the subcuticular layer. The procedure was done under eutrofloxine prophylaxis (Baytril, 5 mg/kg) administered subcutaneously before skin incision. Postoperative pain management consisted of buprenorphine (Buprenex, 0.03 mg/kg subcutaneously) every 12 hours as required.

In one group of rabbits, the aorta was transected and the vehicle PBS/0.1% albumin was delivered (no-tension group; n = 15). In another group of rabbits, a 10 mm ring of aorta was resected and the vehicle PBS/0.1% albumin only was delivered (tension group; n = 14). A third group of rabbits underwent the identical procedure as the tension group animals; however, this group received local delivery of rhVEGF165. Treatment consisted of applying a total of 0.75 μg recombinant human VEGF165 (5 μg/mL; R & D Systems, Minneapolis, MN), which has been used in previous animal experiments by others and our laboratory to the intraluminal surface while performing the anastomosis, and for 10 minutes to the adventitial surface postanastomosis. A total volume of 0.15 mL VEGF was divided between the intraluminal and the adventitial surface (tension + VEGF group; n = 14).

**Tissue Harvesting and Luminal Area Calculation**

At intervals of 1 week (n = 15, 5 of each group), 1 month (n = 13, 5 of no-tension group, 4 each of tension and tension + VEGF group), and 2 months (n = 15, 5 of each group) the animals were weighed and sacrificed. The heart, aortic arch, and descending thoracic aorta were immediately excised. The aorta was transected longitudinally and the inner circumference was measured at the anastomotic site, 1 cm proximal and 1 cm distal of the anastomosis in the aortas harvested at 2 months. The luminal area was calculated accordingly and the relative area change assessed at the anastomotic site compared with the area 1 cm proximal to the site.

**Histology**

The specimens were fixed in 10% buffered formalin. The anastomotic area was divided in 3 portions: the anastomosis itself, the portion including the proximal cross clamp, and the aortic wall between these 2 portions. Tissue was embedded in paraffin and three 4-μm sections were placed on a glass slide and stained with hematoxylin and eosin. The degree of fibrosis, calcification, and chondroid metaplasia was graded 1 to 4 (1 = none; 4 = severe) by a pathologist (SEC) blinded to treatment regimen. The histologic grading system was defined as mild for a single pathologic lesion (lesion not to involve more than 25% of the vessel wall), moderate for multiple pathologic lesions (lesions involve more than 25% but less than 50% of the vessel wall), and severe for diffuse pathologic lesions (lesions involve more than 50% of the vessel wall).

**Statistical Analysis**

Results are expressed as mean±SEM. Statistical analysis of differences was compared by ANOVA and Bonferroni multiple comparison tests. A probability value <0.05 was considered significant.

**Results**

The surgical procedure was performed with a survival rate of 96% (43/45). Two animals, 1 in the no-tension group and 1 in the VEGF-treated tension group, died within 24 hours because of paraplegia. A cross-clamp time of less than 15 minutes was important for survival and to avoid serious neurological deficits. The cross-clamp time was comparable between the no-tension and tension groups (12.4±0.9 versus 13.3±0.8 minutes; n.s.) and between the control tension and tension + VEGF groups (13.3±0.8 versus 12.6±0.8 minutes; n.s.). There was no significant blood loss or other complications related to VEGF treatment. Rabbits of all 3 groups had similar postoperative courses with the percent gain in total body weight ranging from 41% to 47% 2 months after surgery.

**Tension and Vascular Remodeling**

To assess the role of tension on vascular remodeling, we first compared gross and histologic characteristics of the anastomosis in the no-tension and the tension groups. At 1 week, the aorta of both groups appeared grossly similar with only minor irregularities of the aortic endothelial surfaces. However, at 1 month the tension group revealed more marked gross and microscopic changes at the anastomotic site and the adjacent aortic wall when compared with the no-tension group. The histologic grading demonstrated significantly increased degree of fibrosis (mean grade: 2.25±0.3 in no-tension specimens versus 3.5±0.3 in tension specimens; P<0.05) and calcification (2±0.5 versus 3.8±0.2; P<0.05) in the tension group. Both groups revealed multifocal chondroid metaplasia. The gross differences in these 2 groups became even more impressive at 2 months (compare Figure 1A with Figure 1B). The aortic wall of the no-tension group appeared mildly thickened at the suture line, and 2 linear raised lesions were apparent at the site of the former proximal vascular clamp (arrowheads in Figure 1A). In striking contrast, the aorta of the tension group demonstrated a significantly higher degree of remodeling. The entire length of the aortic wall within the vascular clamp-sites was thickened with an irregular cobblestone-like appearance and raised plaques (Figure 1B). Representative histologic sections of all aortas harvested at 2 months from the no-tension group revealed severe adventitial fibrosis infiltrating the vessel media. Small areas of chondroid metaplasia were present within the media (Figure 2A). The histopathological changes were more severe in the tension group. Sections showed band-like regions of dystrophic calcification within the media in all specimens. Intriguingly, islands of mature-appearing cartilage were often observed in direct continuity with the pathologic calcification (Figure 2B).
Influence of VEGF on Vascular Remodeling

As the thoracic aortic end-to-end anastomosis created with tension produced the highest grade of vascular remodeling, we used this model to determine the ability of locally delivered rhVEGF to alter vascular healing. Microscopic evaluation indicated marked differences between the control tension group and the VEGF-treated tension group within 1 week and these findings progressed with time, both in the character and degree of vascular remodeling. At 1 week,

**Figure 1.** Gross appearance of the aortic anastomosis and adjacent aortic wall 2 months after the surgical procedure. (A) The aortic wall of the no-tension group appeared slightly thickened at the suture line, and at the site of the proximal vascular clamp (arrowheads). (B) The tension group aorta revealed severe remodeling. The entire aortic wall within the vascular clamp-site (arrowheads) demonstrated an irregular cobblestone-like appearance. In the area of the suture line itself, a v-shaped retraction of the thickened aortic wall is visible denoting or consistent with anastomotic stenosis (arrows). (C) The VEGF-treated aorta had a smooth luminal surface without gross evidence of calcification. The arrowheads indicate the faint impression of the proximal vascular clamp.

**Figure 2.** Histological findings in the aortic wall adjacent to the anastomotic site harvested 2 months postsurgery. (A) The no-tension specimen had adventitial fibrosis infiltrating the media (arrow). Small islands of cartilage developed within the media (arrowheads) (B) A representative section of the tension group with band-like dystrophic medial calcification (asterisks) and islands of cartilage adjacent to the calcification (arrowheads). (C) The VEGF-treated specimen revealed only mild vascular remodeling with adventitial fibrosis. (A–C) Hematoxylin and Eosin; magnification: × 20; L=lumen.
VEGF-treated specimens showed enhanced healing characterized by only a mild degree of fibrosis. The endothelial cell layer lining the aorta was intact in comparison to the control tension specimens, where the endothelial cell layer was partially denuded and discontinuous. All VEGF-treated aortas harvested at one and 2 months continued to demonstrate significantly less pathology (compare Figure 2C with Figure 2B). This group showed only a mild degree of fibrosis (mean 1.5±0.0), calcification (mean 1.5±0.3) and chondroid metaplasia (mean 1.7±0.3) at 1 month. Microscopic foci of calcification and small islands of chondrocytes were confined to the suture line only in 3 of 5 VEGF-treated animals at 2 months. Grossly, the suture line remained linear with a relatively smooth luminal surface without distortion by calcific plaques. The impression of the proximal vascular clamp is faintly visible as a sign of injury (Figure 1C). In contrast, the tension group specimen revealed severe remodeling within the entire vascular clamp-site (Figure 1B).

**VEGF Reduces Vascular Stenosis**

By 2 months postsurgery, VEGF treatment significantly reduced the luminal stenosis at the anastomotic site to only 3% whereas the tension group specimens revealed a luminal stenosis of 30% (Figure 3; *P*<0.001). A consistent finding in the tension group specimens was a v-shaped retraction of the thickened aortic wall at the suture line itself implying anastomotic stenosis (arrows in Figure 1b). Even in the absence of tension, the luminal area of the anastomotic site had a reduction of 19% and showed a narrowed lumen.

**Discussion**

Recurrent vascular stenosis or recoarctation is the primary indication for further interventions after extended end-to-end anastomosis for repair of coarctation of the aorta in infancy. The majority of recoarctation occurs within the first postoperative year and even in the absence of recoarctation, up to 30% of children develop significant systolic arterial hypertension despite early repair. Arterial physiology studies have shown significant abnormalities in young adults postcoarctation repair. They include arterial dilation in the precoarctation vascular bed and increased vascular stiffness proximal to the site of coarctation repair. Taken together, these observations suggest that underlying pathology persists in the vascular wall postsurgery and this can contribute to significant hemodynamic complications. To investigate the pathogenesis and time course of aortic remodeling, we utilized a rabbit model developed in our laboratory. Unlike the arterial balloon injury model that creates mechanical damage to the endothelium and media our model of aortic end-to-end anastomosis better simulates actual surgical procedures by inducing arterial injury of the entire arterial wall through placement of proximal and distal vascular clamps, transection of the aorta, and the suture line. In addition, while performing the anastomotic procedure, local hypoxia is induced when vascular clamps are in place and when the adventitial vasa vasorum are disrupted during transection of the aorta.

In the present study, we characterized the role of tension on the aortic end-to-end anastomosis and found that tension, created by resection of a ring of aorta, profoundly increased the severity of vascular remodeling. The vascular lesions were characterized by medial calcification with chondroid metaplasia and luminal stenosis was most severe at 2 months postsurgery. This is consistent with the clinical observation by Conte and colleagues who found a significantly higher recoarctation rate in patients with concomitant complete or complex aortic arch obstruction compared with patients with concomitant distal or no aortic arch hypoplasia. The necessity of a more extensive end-to-end anastomosis, meaning an increased distance between the severed aortic ends and thus increased tension on the anastomosis, seemed to result in a higher rate of restenosis. Recently, Vazquez-Jimenez and colleagues addressed the importance of tension on the vascular anatomy by introducing a technique of aortopexy after repair of coarctation to reduce anastomotic tension. However, it is too early to know whether or not this technique is effective in reducing vascular remodeling and thus, decreasing the rate of restenosis.

As hypoxia is known to occur at an arterial anastomosis, we evaluated the impact of the hypoxia-regulated proangiogenic VEGF on vascular healing using the tension model that created the most severe vascular remodeling. A single dose of rhVEGF165, locally delivered, was sufficient to trigger and maintain therapeutically measurable results, i.e., diminishing vascular remodeling and maintaining intact endothelium. Our data revealed a new biological action of VEGF. Topical VEGF diminished calcification and fibrosis in an experimental model of thoracic aortic end-to-end anastomosis with tension and thus significantly reduced the degree of luminal area reduction at the anastomotic site. Recent work has shown that VEGF is capable of inducing endothelial cell survival, which may, in part, be mediated through vascular endothelial cadherin, β-catenin, PI3-kinase, and/or VEGFR-2. Moreover, the endothelium plays a critical role in vascular protection through endothelial cell synthesis of nitric oxide and prostacyclin. Our observation of an intact endothelium in VEGF-treated tension aortas at 1 week and later time points, compared with the damaged endothelium in the control tension aortas, suggests that the endothelial cell may be a target of VEGF in this model (Crawford SE and Seipelt RG, unpublished data). In the
present study it is unclear whether or not VEGF was able to maintain the native endothelial cell layer or induced regeneration of a damaged endothelium within a week, as previously described in a balloon endothelial denudation model, where local administration of VEGF accelerated re-endothelialization leading to marked reduction in intimal thickening. Further studies are needed to clarify protective mechanisms of VEGF on the arterial wall in this model. Nevertheless, preservation of the endothelium appears to be an important protective mechanism to prevent severe vasculopathy after an anortic anastomosis.

**Clinical Implication**

The present study shows that tension on an arterial end-to-end anastomosis results in impaired healing. However, in some clinical situations like repair of coarctation with a hypoplastic transverse aortic arch it is not possible to avoid excessive tension. In this situation it is even more important to extensively mobilize the left subclavian, left carotid and innominate artery as well as the descending thoracic aorta by dividing intercostal collaterals. As a new therapeutic option, a single dose of rhVEGF locally applied at the site of an arterial anastomosis may enhance the healing and thus reduce the risk of severe vessel remodeling or later stenosis. VEGF might be beneficial in other clinical situations like coronary artery bypass grafting or arteriovenous access loops for renal dialysis. In all these situations, one mechanism of failure is stenosis at or adjacent to the anastomotic site, indicating poor healing of the anastomosis with fibrosis or even calcification. Further studies are warranted to determine the optimal concentration and exposure time for this new therapeutic intervention as well as to examine the impact on vascular compliance at the area of the anastomosis.

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**References**

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