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Original article

Antegrade iliac artery stent implantation for the temporal and spatial examination of stent-induced neointimal hyperplasia and alterations in regional fluid dynamics

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Abstract

Introduction: Neointimal hyperplasia remains an important problem after stent implantation. Previous investigations examining vascular responses to stent implantation and effects of drugs have used a retrograde deployment approach that may inadvertently alter the local fluid dynamics surrounding the stent. We present a model of antegrade iliac artery stent implantation that facilitates the analysis of stent-induced alterations in neointimal hyperplasia and wall shear stress in vivo. **Methods:** Stent delivery catheters were inserted through the left carotid artery in anesthetized rabbits (n=37). Catheters were advanced under fluoroscopic guidance to the distal iliac arteries, where the stent was deployed. Hemotoxylin and eosin (H&E) staining of unstented and stented vascular sections was performed 21 days after implantation. **Results:** Selective unilateral stent implantation was successful in 32 of 37 rabbits. No histological abnormalities were observed in the aorta, contralateral unstented iliac, or distal femoral arteries. Neointimal hyperplasia was localized to the stented region. **Discussion:** The model of stent implantation was relatively easy to perform and produced selective neointimal hyperplasia within the stented region without evidence of damage, cellular proliferation, or flow disruption in the surrounding normal arterial vessels. The model will allow detailed examination of the influence of stent implantation on indices of wall shear stress, neointimal hyperplasia, the mechanisms of cellular proliferation in vivo, and their modification by drugs.

Keywords: Iliac artery; Stent implantation; Hyperplasia

1. Introduction

Restenosis after stent implantation remains an important clinical problem (Fischman et al., 1994; Holmes et al., 1998; Serruys et al., 1994; van Beusekom et al., 1998). Stent implantation acutely restores blood flow distal to vascular stenoses but may be associated with cellular damage (Rogers, Tseng, Squire, & Edelman, 1999; Schwartz et al., 1992). Recent data indicate that spatial distributions of shear stress mediate the rate and location of smooth muscle cell migration and proliferation after vascular injury (Liu & Goldman, 2001; Liu, Zhong, & Goldman, 2002), suggesting that stent geometry and deployment ratio may differentially influence restenosis after implantation. We have previously demonstrated that

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design properties unique to the geometry of an implanted stent differentially influence the area of idealized computational vessels subjected to indices of wall shear stress associated with vascular susceptibility to neointimal hyperplasia using 3D computational fluid dynamics models (LaDisa et al., 2003; LaDisa, Olson et al., 2004). The current report presents a methodology that has been developed to test these computational findings experimentally using an antegrade stent implantation model in rabbits.

Previous models of stent implantation in rabbit iliac arteries have used retrograde stent placement performed through the femoral artery (Burgio, Martini, Avanzini, Paulli, & Rosso, 1984; Garasic et al., 2000; Herdeg et al., 2003). However, this retrograde model may introduce alterations in the localized flow environment distal to the stent, which may subsequently influence cellular proliferation within the stented region, and is not representative of typical catheter entrance to regional vascular lesions. We present an antegrade model of stent implantation that can be used to investigate spatial and temporal alterations in neointimal hyperplasia within the interventional region in the absence of flow disturbances introduced by the method of deployment. We have preliminarily reported the use of this model to compare temporal alterations in vascular histology with concomitant alterations in indices of wall shear stress after stent implantation (LaDisa, Warltier et al., 2004). These data suggest a high correlation between areas of low wall shear stress and neointimal hyperplasia within the stented zone. We also demonstrate how the model can also be employed to analyze the local expression of molecular mediators that contribute to cellular proliferation within the stented region to elucidate mechanisms of restenosis.

2. Methods

2.1. Use and care of animals

All experimental procedures and protocols used in this investigation were approved by the Animal Care and Use Committee of Marquette University and the Medical College of Wisconsin. Furthermore, all conformed to the *Guiding Principles in the Care and Use of Animals* of the American Physiological Society and the *Guide for the Care and Use of Laboratory Animals* as outlined by the National Institutes of Health (7th ed. Washington, DC: Nat. Acad. Press, 1996).

2.2. Antegrade iliac artery stent implantation

Male New Zealand white rabbits (n=37) weighing approximately 3.5 kg were obtained from New Franken Researchers (New Franken, WI). The rabbit iliac artery was selected for stent implantation because the diameter and length of this vessel closely approximates the size of stents frequently used in clinical settings (3.0 and 16 mm, respectively). The absence of axial curvature in these arteries and the ability to obtain experimental (stent) and chronic control data in the contralateral iliac arteries of a single animal are also important benefits of this model. Stent implantation was performed under sterile conditions using portable fluoroscopic imaging (OEC, GE Medical Systems, Milwaukee, WI). Rabbits were anesthetized intravenously through the right marginal ear vein with a mixture of xylazine (2.2 mg/kg) and ketamine (22 mg/kg) and intubated using a 3.0-mm pediatric endotracheal tube. Anesthesia was maintained with 1% to 2% isoflurane in oxygen, and rabbits were allowed to breathe spontaneously. Body temperature was controlled at 37 °C through the use of a heating pad.

An incision was made above the left carotid artery and the vessel dissected free. A 5F introducer sheath (Avanti+, Cordis, Miami, FL) was inserted into the vessel, through which a fluid-filled 5F JR3.5-5 softip guide catheter, preloaded with a guide wire (Entre II; Boston Scientific, Maple Grove, MN), was advanced from the carotid artery into the distal descending aorta. The guide wire was then advanced to the proximal portion of the right iliac artery. The contralateral iliac artery was not instrumented and served as a control. The guide catheter was carefully removed while the wire remained in place. The stent delivery system was advanced to the distal portion of the iliac artery using the guide wire and the stent was deployed to a pressure of 5 atm to achieve a stent-to-artery-size ratio range of 1.1 to 1.2:1 (Garasic et al., 2000). Intravenous heparin (100 U/kg) was administered immediately after stent deployment (Rogers & Edelman, 1995). Successful deployment was verified by angiography, and the stent delivery catheter was removed. The left carotid artery was ligated, the incision above the carotid artery closed in layers, and the rabbit allowed to emerge from anesthesia. Intramuscular buprenorphine (0.05 mg/kg, BID) was used for the treatment of postoperative pain. Rabbits also received antibiotic prophylaxis (enrofloxacin, 5 mg/kg) for 4 days after stent implantation and aspirin in their drinking water (20 mg/day) for the duration of the protocol (Rogers & Edelman, 1995).

2.3. Histological analysis

Additional rabbits (n=12) were used for histological analysis of stented and unstented arteries. Rabbits underwent iliac artery stent implantation as described above and were sacrificed 21 days after deployment. On the day of sacrifice, rabbits were anesthetized as previously described, the chest opened by a midline sternotomy, and the pericardium incised. The apex of the left ventricle was perfused at a mean arterial pressure of 100 mm Hg with lactated Ringer's solution, followed by Carnoy's solution (Rogers, Edelman, & Simon, 1998) while the rabbit was simultaneously exsanguinated through a right atrial incision. The proximal and distal ends of the iliac arteries were identified, and the vessels were