Trans-Iliac Rat Aorta Stenting: A Novel High Throughput Preclinical Stent Model for Restenosis and Thrombosis

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Background. Currently, preclinical stent development requires elaborate large animal models, which are time consuming and expensive. We herein report a high throughput rat aorta stenting model which could provide a rapid and low-cost platform for preclinical stent development.

Methods. A total of 86 metal stents (316L stainless steel 13 mm, VasoTech, Inc.) coated with poly (D, L-lactide-co-glycolide)/amorphous calcium phosphate (PLGA/ACP) copolymer were pre-mounted on 1.5 mm × 15 mm balloon catheters and were implanted into aspirin treated Sprague-Dawley rats (500–700 g) initially using either direct placement in the abdominal aorta (group A, $n = 7$) or a trans-iliac approach (cut-down, group B, $n = 79$). The surviving rats were sacrificed at 1, 2, 4, and 12 wk post-implantation and the stented arteries were analyzed histopathologically.

Results. Four rats died in group A and nine rats died in group B within 48 h post-stent implantation (mortality: 57% *versus* 11%, $P < 0.05$). All animals that died had stent thrombosis/paralysis with visible thrombus on necropsy. Histologically, neointimal growth peaked at approximately 4 wk post-implantation.

Conclusion. This result suggests that human-sized stents can be successfully implanted into the rat aorta *via* iliac artery insertion with a significantly higher survival rate than trans-aorta implantation. The model system allows rapid (4–12 wk) assessment of stent biocompatibility with mortality/paralysis used as an indicator of stent thrombosis. © 2011 Elsevier Inc. All rights reserved.

Key Words: iliac artery; aorta; stent; restenosis; thrombosis.

INTRODUCTION

In-stent restenosis (ISR) has been one of the most serious complications since the introduction of stent technology [1]. Though the development of drug-eluting stents (DESs) was a major breakthrough as a potential solution for ISR, ISR in high risk patients with small vessels, diabetes, and long segments of diffusely diseased arteries still remains unacceptably high (30%–60% in bare metal stents and 6%–18% in drug coated stents) despite DES implantation [2–4]. Therefore, for the development of next generation of DESs, a high throughput animal model is essential for stent evaluation before clinical trials.

The rabbit iliac artery and the porcine coronary models of ISR are widely used for stent evaluation, however, these models are costly with numerous well documented limitations [5]. Stenting of the rat carotid artery or aorta as a model to evaluate stents has also been reported extensively [6–9]. However, due to the limitation of rat carotid artery size, a specially designed miniature stent with delivery system is required, and therefore the data generated from this system are compromised. For the rat aorta stenting model in which some preliminary results on the successful coronary stent placement have been reported [10–12], a human sized stent and delivery system can be utilized as the size of rat thoracic

arteries is close to that of the human coronary artery (approximately 2.5 mm). Currently, two approaches to rat aorta stent placement have been reported: through the carotid artery and direct abdominal aortic insertion. Insertion through the carotid artery to the aorta will not block blood flow, which avoids aortic thrombosis, but the procedure requires a highly-skilled physician to successfully complete. Insertion through an incision in the lower abdomen, although technically easy, requires interruption of the aortic blood flow, which may cause acute thrombosis. In this study, we present a novel approach to abdominal aortic stenting utilizing the common iliac artery, which results in minimal interruption in aortic blood flow. Additionally, animal survival rates were compared between our approach and direct abdominal aortic incision stenting.

FIG. 1. Animal mortality of two surgical methods. Trans-abdomial aorta (group A, $n = 7$) and trans-iliac artery (group B, $n = 79$).

MATERIALS AND METHODS

Animal Protocol

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Beth Israel Deaconess Medical Center, Harvard Medical School, and performed in accordance with the protocol approved by IACUC. Specific pathogen-free, male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 500–700 g were fed aspirin-incorporated food (5 mg/kg/d, Bio-Serv, Frenchtown, NJ) commencing 3 d before the surgery and maintained through the entire study period. All polymer coated stents (1.5 mm ×13 mm) were premounted on VasoTech miniature balloon catheters (1.5 mm × 15 mm, VasoTech, Inc.) and sterilized with Ethylene Oxide (ETO) for 60 min before implantation.

All animals were fasted for 12 h before the surgery. After the animal was fully anesthetized with constant inhalation of a mixture of oxygen/isoflurane (1.5:2 pressure/pressure) and heparinized based on the animal weight with a standard dose (100 IU/kg i.m.) of heparin, the abdominal aorta and left iliac artery were exposed. The stents were inserted into the abdominal aorta 10mm above the bifurcation through either abdominal aorta incision (group A, $n = 7$) or left iliac arterial incision (group B, $n = 79$) by arteriotomy and direct placement, and were deployed by inflating the balloon catheter to 10 ATM pressure for 30 s. The balloon catheter was deflated to maintain negative pressure for 30 s. The process was repeated three times to fully deploy the stent. The deflated catheter was then withdrawn slowly while leaving the stent in place. The arteriotomy was then repaired with 7-0 bioabsorbable suture. All animals were allowed to recover and returned to the animal care facility where they continued receiving antiplatelet therapy for the entire study period. Antibiotics (gentamycin, 50 mg/kg, i.m., Abbott Laboratories, Abbott Park, Chicago, IL) were given to all animals for 3 d following the surgery. Postoperative analgesia was administered, as needed, at the discretion of the attending veterinarian.

Animals were monitored daily with body weight changes and signs of thrombosis (paralysis of lower extremities) post-stent implantation through the entire study period. If there was any sign of thrombosis, the animals were scarified and necropsied immediately. The surviving rats were sacrificed at predetermined time points of 1, 2, 4, and 12 wk. During the necropsy, the rats were first perfused with heparinized saline under deep anesthesia at 80 mmHg pressure through the left ventricle until the perfusate from right atria was clear of blood and were then continuously perfused with 4% buffered formalin for 30 min to fix the stented arteries. The stented arteries were then removed and stored in 4% buffered formalin for further pathologic
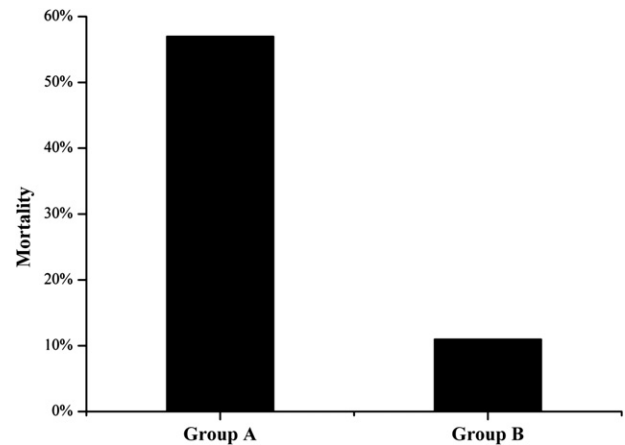
processing. For the pathologic analysis, the stented arterial tissue was plastic embedded, processed and stained with hematoxylin and eosin (H and E) as previously described by Quentin et al [13]. Briefly, the fixed stented arteries were dehydrated and embedded in methyl-methacrylate (Technovit 9100 New kit; Heraeus Kulzer GmbH, Germany) following the protocol supplied by the manufacturer. Three 200 μm-thick cross-sections were cut from the proximal, middle, and distal points of the resin-embedded specimen using a low-speed precision saw (IsoMet 1000; Buehler, Lake Bluff, IL). All sections were then ground down to approximately 10–20 μm using silicon carbide abrasive papers with decreasing grit (320/600/1200) on a variable speed grinder-polisher (EcoMet 3000; Buehler). The polished cross-section was rehydrated and stained with H and E (Fisher Scientific Company, LLC, Kalamazoo, MI).

The morphopathologic analysis was completed by an investigator blinded to the treatment groups. Each section was examined under a microscope (Olympus BX60; Olympus Co., Tokyo, Japan) with a camera system (Hitachi HV-C20 3-CCD color camera; Hitachi Denshi, Ltd., Koganei, Japan) and computerized histomorphometrically by an image analysis system (Image-Pro Plus ver. 4.5; Media Cybernetics, Inc., Bethesda, MD). The cross sectional areas of the lumen, neointima, media, and adventia were measured. Percentage of restenosis was calculated as follows: the area within the internal elastic lamina (IEL) was considered the normal lumen area. The IEL area
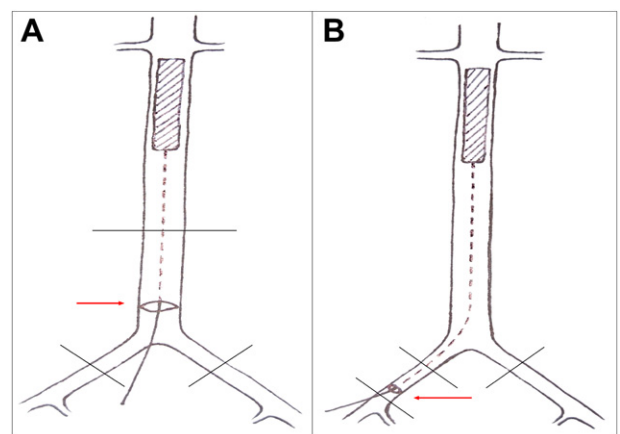

FIG. 2. Schematic of (A) trans-abdominal aorta, and (B) trans-iliac artery. Arrows indicate the aortic/arterial incisions.