Characterization of Vascular Injury Responses to Stent Insertion in an Ex-vivo Arterial Perfusion Model

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ABSTRACT

Purpose: To develop an ex-vivo arterial perfusion model to evaluate vascular responses to bare metal stents (BMS) and drug-eluting stents (DES) in porcine carotid arteries.

Materials and Methods: Porcine carotid arteries with BMS or DES were cultured under hemodynamic stimuli for 24 hours and 72 hours. Vascular responses of arteries with stents were assessed by cellular functionality and gene expression and compared with a noninjured (NI) control group at each time point. Cellular functionality was confirmed with sequential dosing of norepinephrine (NE), acetylcholine (ACH), and sodium nitroprusside (SNP). QuantiGene (Panomics, Fremont, California) branched DNA (bDNA) assay was used to evaluate gene expression of endothelial cell (EC) and smooth muscle cell (SMC) biomarkers and compare it with responses of in-vivo arteries with stents. Bromodeoxyuridine (BrDU) stain was also used to detect cellular proliferation in the ex-vivo arteries with stents.

Results: EC relaxation and SMC contraction in response to vasoactivators indicated the arteries remained viable and functional for at least 72 hours in culture. SMC-dependent contractility and EC-dependent relaxation were lower in arteries with stents compared with NI arteries. Greater SMC proliferation was observed in BMS arteries compared with DES arteries. Cellular proliferation, EC function, and SMC marker expression at acute time points were similar between both models suggesting that the ex-vivo arterial model can provide comparative predictions of stent injury in vivo.

Conclusions: The ex-vivo arterial perfusion model can be used as a quick and less costly (than current in-vivo and some in-vitro perfusion testing models) approach for evaluating the vascular responses to various stent design parameters (eg, strut thickness, strut width).

ABBREVIATIONS

ACH = acetylcholine, bDNA = branched DNA, BMS = bare metal stent, BrDU = bromodeoxyuridine, CTGF = connective tissue growth factor, DAPI = diamidino-2-phenylindole, DES = drug-eluting stent, EC = endothelial cell, eNOS = endothelial nitric oxide synthase, MMP2 = matrix metalloproteinase-2, NE = norepinephrine, NI = noninjured, PCNA = proliferating cell nuclear antigen, PECAM1 = platelet endothelial cell adhesion molecule-1, PLAU = urokinase plasminogen activator, SMC = smooth muscle cell, SNP = sodium nitroprusside, VE-cadherin = vascular endothelial cadherin

In-vitro, ex-vivo, and in-vivo models have been developed to understand better the vascular biology and pathology of atherosclerotic disease and the effect of therapeutic agents.

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The ex-vivo tissue culture system has been described as an alternative model to in-vivo animal and in-vitro cell culture models for studying vascular responses to biomechanical and biochemical factors (1). The ex-vivo model uses arterial tissues, allowing for the evaluation of cellular interactions and biologic responses in the three-dimensional architecture of the tissue under controlled physiologic conditions (pH, flow rate, pressure, and shear stress). Yamawaki et al (2) and Wright et al (3) discussed the applications of similar ex-vivo tissue culture models to study the proliferative changes in vascular smooth muscle cells (SMC), an important phenomenon in atherosclerosis and neointimal formation, in response to the absence or presence of serum (culture media). George et al (4) investigated the role of

growth factors such as platelet-derived growth factor in neointimal formation using a saphenous vein culture model.

The ex-vivo tissue culture model has also been used to study the physiologic mechanisms of vascular tissue and other smooth muscle organs (5). Voisard et al (6) treated renal arteries with fetal bovine serum for up to 3 weeks to develop restenosis models. The ability of the tissue culture model to maintain cell-cell interactions compared with in vitro models was showcased in the article by Ligush and Labadie (7), which evaluated the role of the vascular endothelial layer and nitric oxide in mediating vasodilation. The model tissue culture has not been widely used, however, to understand the cellular functionality and proliferation in response to injury induced by stents to the arterial vasculature. Yazdani and Berry (8) investigated the effect of stent design on SMC proliferation in ex-vivo porcine carotid arteries using carotid self-expanding stents.

Implantation in coronary arteries of bare metal stents (BMS) and drug-eluting stents (DES) has been the current procedure of choice for treating atherosclerotic lesions in patients as a mechanical means to maintain artery lumen patency after balloon angioplasty. To our knowledge, the ex-vivo tissue culture model has not been used to evaluate the influence of coronary stent design and polymer and drug coating on vascular responses. This model provides a less costly approach than current in-vivo and some in-vitro perfusion models for evaluating stent parameters in controlled, physiologic settings. It also provides the opportunity to refine these parameters before in-vivo evaluations.

The purpose of this study is to develop and use the ex-vivo tissue culture model to evaluate vascular responses to stenting at acute time points (24 hours and 72 hours) using two commercially available stent delivery systems, the MULTI-LINK MINI VISION (Abbott Vascular Inc., Santa Clara, California) BMS and XIENCE V (Abbott Vascular Inc, Santa Clara, California) DES. Vascular responses after stent implantation were evaluated using*(i)* vasomotor functionality testing to measure the contraction and relaxation of the arteries,*(ii)* QuantiGene (Panomics, Fremont, California) branched DNA (bDNA) gene expression assay to measure the levels of functional endothelial cell (EC) and SMC biomarker expression, and*(iii)* immunohistochemical staining to detect differences in cellular proliferation induced by implantation of BMS and DES.

MATERIALS AND METHODS

Tissue Harvest and Shipping

Porcine common carotid arteries were harvested from 110 kg ± 10, 6- to 7-month-old male and female swine from an abattoir (Pel-Freez Biologicals, Rogers, Arkansas) and shipped overnight on wet ice in sterilized 50-mL tubes containing low-glucose Dulbecco's Modified Eagle Medium (Invitrogen, Carlsbad, California) with 1.4% antibiotic-antimycotic (penicillin-streptomycin; Invitrogen, California) and 0.1% gentamicin (Sigma-Aldrich, St. Louis, Missouri). Animal care at this facility complied with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health).

Tissue Handling—Cleaning and Leak Testing

On arrival, the arteries were dissected free of the surrounding adipose and connective tissues. Side branches were ligated with sterilized surgical sutures to prevent leaking during the perfusion period. The arteries were tested for leaks by pressurizing with air at very low pressure while submerged in preparation media (Dulbecco's Modified Eagle Medium with 1.4% antibiotic-antimycotic and 0.1% gentamicin) (9). Arteries (approximately 65–80 mm in length and 3.0–3.5 mm in inner diameter) were rinsed three times by placing each individual artery in a sterilized test tube containing preparation media on a shaker (Lab-Line Instruments, Inc, Melrose Park, Illinois). All arteries were set up for culture in the ex-vivo perfusion system on the same day.

Endothelial Cell Viability and En Face Preparation

Randomly selected arterial segments (5–7 mm in length) before and after culture were assessed for EC viability using a modified Häutchen en face procedure (9). Briefly, the segments were placed in a six-well cell culture plate (VWR, Bridgeport, New Jersey) containing freshly prepared 2 μM/mL of propidium iodide (Calbiochem, San Diego, California) to stain for dead ECs and incubated on a shaker, for 10 minutes at 37°C, 5% CO_2. The tissues were rinsed with phosphate buffered saline (Invitrogen, Carlsbad, California) followed by en face preparation.

To view all ECs, the cellulose polyacetate paper was counterstained with 4,6-diamidino-2-phenylindole (DAPI) in mounting medium (Vector Laboratories, Burlingame, California), placed on a glass slide with a glass cover. Live and dead ECs were imaged using a fluorescent microscope (Nikon, Melville, New York) under dual excitation/emission wavelengths of 330–380 nm for DAPI and 528–553 nm and Simple PCI software (Hamamatsu Corp, Sewickley, Pennsylvania).

Perfusion System Setup and Media Composition

The ex-vivo perfusion system, described in detail by Conklin et al (9), was modified in this study. The artery was housed in a single, polycarbonate chamber, which was connected to a closed, circulating flow loop. The cell culture medium surrounding the artery was also circulated through the arterial lumen with a peristaltic pump (Masterflex, Vernon Hills, Illinois). The transmural pressure, flow rate, longitudinal stretch, and shear stress were independently controlled in each system.