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Direct Left Ventricle–to–Coronary Artery Stent Restores Perfusion to Chronic Ischemic Swine Myocardium

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ABSTRACT

Background. Direct left ventricle (LV)-to–coronary artery shunts (VSTENT) have been proposed as an alternative means of myocardial revascularization. The goal of this study was to examine quantitative changes in myocardial perfusion and possible mechanisms of revascularization with an LV-to-coronary shunt.

Methods. Ameroid occluders were implanted on the proximal left anterior descending coronary artery (LAD) of 6 pigs to create chronic ischemia. Four weeks later, a VSTENT was placed to directly connect the distal LAD with the LV chamber. Animals survived for an additional 3 weeks and received periodic bromodeoxyuridine (BrdU) injections to identify dividing cells to identify and quantify angiogenesis. Regional myocardial perfusion (RMP) was measured with color microspheres under adenosine vasodilatory stress before and 3 weeks after VSTENT implantation. Vascularity was assessed histologically by an overall vascularity index and a growth index reflecting the density of BrdU-positive vascular cells.

Results. Three weeks after VSTENT placement, RMP improved from 38.4% ± 19.6% of non-ischemic flow to 86.8% ± 13.7% in treated animals ($P < .05$). This benefit was accompanied by histological evidence of increased vascularity and vascular proliferation. Four of 5 animals had patent and functional devices at the end of the study.

Conclusion. Chronic VSTENT placement improves RMP and may promote arterial remodeling in chronically ischemic porcine myocardium.

Dr. Yi and Dr. George contributed equally to this manuscript.

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INTRODUCTION

Despite advances in medical care, coronary artery disease in industrialized countries continues to cause major morbidity and mortality, with more than 1,500,000 people undergoing percutaneous intervention in 2002 worldwide [American Heart Association 2001]. However, an estimated 12% of patients with coronary disease are not candidates for conventional revascularization, either through coronary artery bypass grafting or percutaneous coronary interventions [Mukherjee 1999], due to recurrent coronary stenoses, failed bypass grafts, poor target vessels, or problematic aortic anastomoses. Historically, these patients have had few options other than cardiac transplantation. However, a method to direct blood from the left ventricle (LV) to the coronary arteries would be ideal to overcome these limitations. Thus, ventricular sourcing, or LV-to–coronary artery shunting (VSTENT), has been proposed as an alternative means of myocardial revascularization [Suehiro 2001; Yi 2005].

Ventricular sourcing may be an ideal method of revascularization in these patients with diffuse coronary disease or small coronary targets. Normally, the heart is primarily perfused during diastole when coronary artery resistance is low. The closed aortic valve maintains a high blood pressure during diastole to ensure a large pressure gradient that drives myocardial perfusion. Recurrent stenoses secondary to atherosclerotic disease or failed grafts reduce the pressure gradient required for coronary perfusion. With ventricular sourcing, flow is restored to the myocardium via a low-resistance shunt supplied during systole. Reversal of flow, from the coronaries to LV, is seen even during diastole, which is hypothesized to have multiple beneficial effects, such as reducing stasis and deposition long term [Emery 2004]. A polytetrafluoroethylene-covered stent (VSTENT; Pericardia, Merrimack, NH, USA) inserted from the left anterior descending coronary artery (LAD) to the LV has been shown to acutely provide up to 70% of normal LAD perfusion in preliminary animal studies and restore cardiac function as well [Suehiro 2001; Yi 2005].

At this time, it is unknown if such shunts can improve long-term perfusion in chronically ischemic myocardium. Furthermore, the mechanisms by which perfusion is increased have not been clearly elucidated. It is theorized that angiogenesis is promoted in myocardium supplied by ventricular sourcing, contributing to improvements in perfusion. The objective of the present study was to evaluate changes in regional myocardial perfusion and quantify coronary angiogenesis after placement of the VSTENT in a chronic ischemia porcine model.

MATERIALS AND METHODS

Studies were performed in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. This study was approved by the Institutional Animal Care and Use Committee of Columbia University.

Study Design and Surgical Preparation

Eight Yorkshire domestic pigs of either sex at starting body weights of 40 to 45 kg were used in this study. The study consisted of 3 surgeries: 1) an ameroid occluder was placed around the LAD to create chronic ischemia; 2) 4 weeks after ischemia was established, the VSTENT device was implanted (n = 6) or sham thoracotomy performed (n = 2); and 3) a terminal experiment was performed with excision of the heart 3 weeks after the device implantation. Anesthesia was induced with ketamine (20 mg/kg, IV) and was maintained with 1.5% to 2.0% inhaled isofluorane. At the first surgery, a left thoracotomy was performed in the third intercostal space. The proximal LAD artery was isolated, and an ameroid constrictor (Research Instruments SW, Escondido, CA, USA) with internal diameter of 2.75 mm was placed to induce myocardial ischemia over time. The hygroscopic ameroid material slowly swells, gradually leading to complete closure of the coronary artery around 10 days after placement, with minimal infarction [Oesterle 2000]. In addition, a Tygon catheter (Cardiovascular Instruments, Wakefield, MA, USA) was implanted in the descending thoracic aorta, and a second catheter was inserted in the left atrial appendage for colored microspheres (CMS) administration. The chest was closed in layers after placement of a chest tube. All catheters were tunneled subcutaneously and housed using a specially designed backpack. Antibiotics were given as necessary postoperatively. The animals were allowed to recover fully from the surgery.

The second surgery was performed 4 weeks after the ameroid constrictor implantation. Aspirin (325 mg) and Plavix (75 mg) were given orally for 3 days prior to the second surgery and maintained until termination. The same anesthesia procedure was used as in the first surgery. Coronary angiography was performed via one side of the femoral artery to verify LAD occlusion due to ameroid occlusion and the absence of distal LAD flow. A partial midline sternotomy was then performed to allow adequate access to the heart and to avoid tissue adhesions caused by the first surgery. The pig was heparinized with 5000 U intravenously. An Octopus tissue stabilizer (Medtronic, Minneapolis, MN, USA) was used to minimize heart motion during the procedure. The VSTENT device was implanted between the coronary artery and LV via a coronary arteriotomy by directly puncturing the ventricle. A piece of pericardial patch was used to close the coronary arteriotomy. The chest was closed, and the pigs were then allowed to recover and survive for an additional 3 weeks. At the end of 3 weeks, repeat myocardial perfusion studies were performed, and the animals were sacrificed. The 2 control animals that had sham surgery were observed for a total of 3 months instead of 3 weeks after the second surgery, followed by sacrifice. This was allowed to fully recognize any late effects of re-collateralization.

Assessment of Myocardial Perfusion

Four weeks after ameroid constrictor placement, myocardial blood flow was measured at rest and during maximal vasodilation with adenosine infusion. CMS (Dye-Trak; Triton Technology, San Diego, CA, USA) were infused into the left atrium through the previously implanted left atrial catheter (2 mL, 6×10^6 spheres). Blood samples were then obtained just prior to microsphere injections by constant rate blood withdrawal (7 mL/min for 2 minutes) from the implanted aortic catheter using a syringe pump. Adenosine vasodilatory stress was then infused in titrated doses to induce a 25% decrease in mean aortic pressure followed by infusion (and aortic reference sample withdrawal) of a second set of CMS. Myocardial blood flow was assessed before device placement (chronic ischemia), immediately following device placement (acute VSTENT), and 3 weeks after device implantation (chronic VSTENT). To assess myocardial perfusion, whole-heart perfusion maps were created based on CMS data, as previously described [Roethy 2001]. At the conclusion of each experiment, the heart was cut into 5 layers (A through E, base to apex), and each layer was then cut into 8 pieces (except for the apex, which was cut into 4) resulting in a total of 36 transmural segments. Blood flow was calculated from the CMS data according to standard techniques [Kowallik 1991; Roethy 2001; Suehiro 2001]. Briefly, ventricular tissue digestion was achieved using a high-grade vacuum filtration filter after mixture with a 4 M KOH solution containing 2% Tween 80. Colored dye was recovered using 100 μL of toluene-dimethylformamide and multiple centrifugation repetitions. Finally, the photometric absorption of each dye solution was measured using an ultraviolet/visible spectrophotometer, and individual dye components were resolved using a matrix inversion technique from the composite spectrum. Perfusion was expressed as single spectra absorption after a linear regression analysis. Whole-heart myocardial perfusion, expressed as percentage of flow in the non-ischemic zone (posterior wall of LV), was then plotted as a function of segment number, 1 through 36. Regional myocardial perfusion of the ischemic zone (anterior wall of LV) was also expressed as a percentage of flow in the non-ischemic zone (posterior wall of LV).

Histology/Pathology

To provide an index of cellular proliferation at multiple time points following the device implantation, all treated

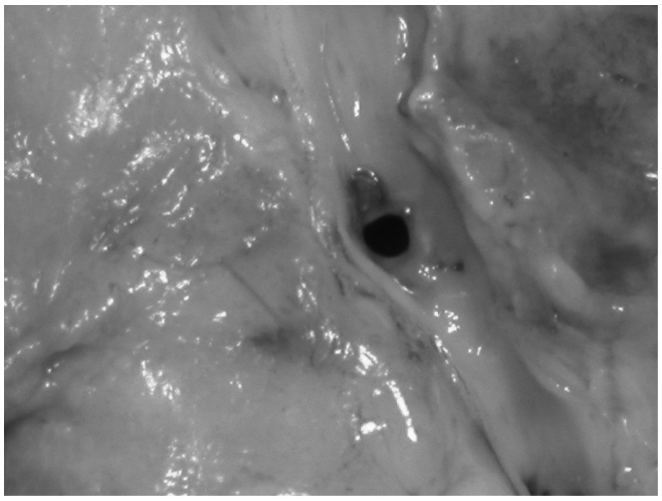

Figure 1. A view of the VSTENT device from inside the left ventricle 3 weeks after implantation. No thrombus or neointimal hyperplasia is seen at the inlet orifice.

animals received postoperative bromodeoxyuridine (BrdU) (25 mg/kg body weight) subcutaneous injections on days 1, 3, 7, 14, and 21 after device implantation surgery. BrdU, a thymidine analogue marker, was used to identify activating proliferating cells in the S phase in vascular endothelium. At the terminal experiment after the heart excision, 2 transmural blocks were removed from the anterior wall in the region previously perfused by the occluded LAD (the ischemic region) and later perfused by the device, as well as blocks from the posterior wall (control area), for Masson's trichrome and BrdU analysis. In addition, a piece of intestine was removed from each animal and evaluated for BrdU staining as a reference.

Histology samples taken from the ischemic and nonischemic areas of the LV were examined for vascular density using trichrome staining at experiment termination. An overall vascularity score comparing the ischemic and nonischemic areas was created by a blinded observer from these slides using the following index:

1 = No change between ischemic and nonischemic regions, otherwise normal.
2 = Appearance of new small to intermediate blood vessels.
3 = Appearance of new large blood vessels.

Samples were subsequently graded for vascular proliferation according to a semiquantitative overall growth index [Roethy 2001].

1= No BrdU staining of smooth muscle or endothelium.
2 = BrdU staining mainly confined to capillaries and interstitium.
3 = BrdU staining of small vessels.
4 = BrdU staining (>2 cells) in large arteries.
5 = Abundant BrdU staining in large arteries.

Statistics

All data are presented as means and standard deviation. Statistical comparisons were performed by independent *t* testing and within-group analysis was performed using paired *t* testing, with a $P < .05$ considered significant. All calculations were performed using SPSS software (Chicago, IL, USA).

RESULTS

Safety and Long-Term Patency of the VSTENT

Five of the 6 animals survived to completion of the experiments. One animal died during the recovery of the VSTENT

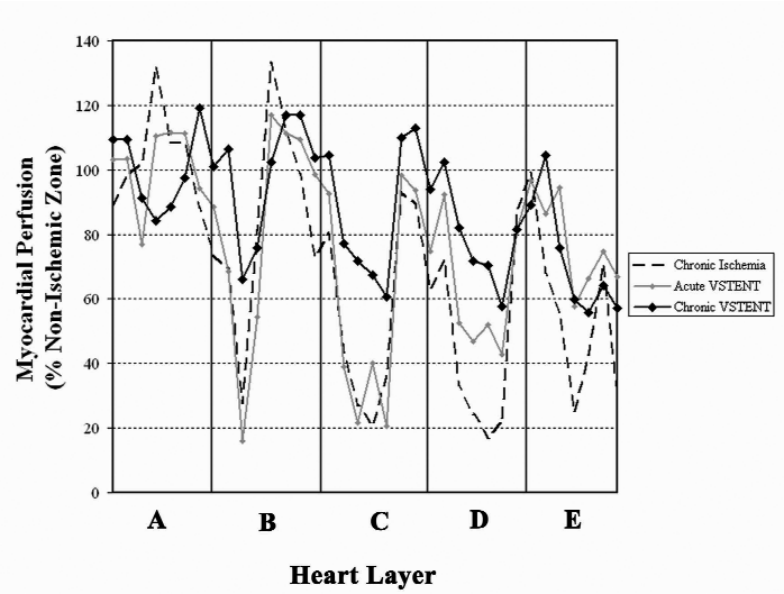

Figure 2. Myocardial perfusion as a percentage of nonischemic zone flow before and after VSTENT placement per heart layer (base to apex, A to E) for one treated animal.

Table 1. Regional Myocardial Perfusion under Adenosine Stress (% of Nonischemic Zone Perfusion)*

	Chronic Ischemia	Chronic Ischemia + Acute VSTENT	Chronic VSTENT
VSTENT	38.4 ± 19.6	56.8 ± 28.2†	86.8 ± 13.7‡

*All values are mean ± standard deviation.

†P = .179 versus chronic ischemia.

‡P = .013 versus chronic ischemia.

Table 2. Bromodeoxyuridine Staining and Vascularity Index after 3 Weeks VSTENT Implantation*

	Ischemic Zone	Nonischemic Zone
Bromodeoxyuridine staining	3.00 ± 1.0†	0.33 ± 0.58
Vascularity index	2.33 ± 0.58†	1.00 ± 0.00

*All values are mean ± standard deviation.

†P < .05 versus nonischemic zone.

device after the second surgery. Four of 5 animals had patent and functional devices at the end of the experiment. Figure 1 shows a sample image taken from an animal with a patent device for 3 weeks. The seating of the VSTENT on the bottom of the coronary artery is optimal, and the channel remained patent and free from thrombus. No technical complications, hemorrhage, or coronary vessel damage was encountered during implantation in any animal. No adverse side effects relating to device insertion or function were seen in the postoperative period. The one animal with an occluded device had no visible thrombus, but formed a thin endocardial lining covering the device opening to the LV.

Myocardial Perfusion

Global perfusion was increased in all animals with a patent VSTENT device. Figure 2 shows an example of one treated animal's data for whole-heart myocardial perfusion throughout the course of the experiment; all other treated animals had highly similar results. As shown, there is evidence of only mildly increased flow to all layers of the hearts immediately after device placement (acute VSTENT), from base to apex. However, global perfusion to all regions increased greatly 3 weeks after device placement (chronic VSTENT).

Regional perfusion, ie, flow to ischemic LV, similarly showed greater improvements after 3 weeks of device placement compared to flow in nonischemic posterior wall LV. Flow from nonischemic LV zones was used as a reference value for each animal under the assumption that flow to this region would not change throughout the course of the experiment. Regional perfusion under adenosine vasodilatory stress was diminished to 38% of flow to nonischemic zones after ameroid occlusion (chronic ischemia), but was restored to 56% immediately after device implantation (acute VSTENT). Furthermore, after 3 weeks of revascularization using the VSTENT (chronic VSTENT), regional myocardial perfusion was restored significantly, up to almost 87%. See Table 1 for a summary of regional perfusion data. In contrast, control animals showed a slight decline in regional perfusion from baseline to 3 months, from 50.2% ± 5.5% to 47.8% ± 2.0%.

Histology

Improvement of overall vascularity (1.00 ± 0.00 in control zone versus 2.33 ± 0.58 in ischemic zone, P < .05) and marked vascular proliferation (BrdU) (0.33 ± 0.58 in control zone versus 3.00 ± 1.00 in ischemic zone, P < .05) was seen histologically 3 weeks after device placement (Table 2). Figure 3 shows examples of trichrome and BrdU staining in ischemic (supplied by VSTENT) and nonischemic (control) regions. Evidence of new small vessel growth is found on the ischemic LV anterior wall after device implantation. In contrast, there was no positive staining for BrdU shown in the myocardium from the posterior (control) wall.

DISCUSSION

The number of patients with coronary artery disease undergoing either percutaneous coronary interventions or coronary bypass surgery continues to increase in the U.S. A subset of patients, despite maximal medical therapy, will manifest progressive ischemia with viable myocardium and would benefit from some form of revascularization [Almeda 2003], but are not candidates for traditional revascularization [Yamamoto 1998; Frazier 1999; Kantor 1999; Yeung 1999; Oesterle 2000; Stone 2002,]. Ventricular sourcing may be an alternative method of revascularization for these patients to provide long-term perfusion.

In this study, a VSTENT, or direct LV-to-coronary artery shunt, was implanted after chronic ischemia was produced by occlusion of the LAD. Patency of the VSTENT was maintained for 3 weeks on standard antiplatelet therapy. Myocardial perfusion was restored to 87% of baseline after 3 weeks, and BrdU staining suggested angiogenesis in the ischemic region supplied by the VSTENT.

In our previous study, ventricular sourcing improved myocardial perfusion in acutely ischemic myocardium to 70% of normal perfusion [Suehiro 2001; Yi 2005]. In the current chronic study, 87% of normal myocardial perfusion was recovered under adenosine stress. The full extent to which perfusion was restored in chronically ischemic myocardium was not seen immediately after device implantation, but occurred after 3 weeks of restored perfusion. The findings suggest that long-term effects of hypoperfusion may have led to a regression of normal vascular connections between conduit vessels and the microvasculature, which limits its ability to normally distribute flow on an endothelial level when flow to the myocardium is acutely restored. Eventual improvement in reperfusion via VSTENT may require re-establishment of these microvascular connections.

Direct LV shunting through the patent VSTENT supplies the myocardium with a fresh source of blood through each cardiac cycle in systole. However, the stimulation of angiogenesis and arteriogenesis that occurs after long-term VSTENT use may represent an additional contribution to the overall function of ischemic myocardium. In this study, increases in vascularity were seen on histologic staining, and regional staining showed a

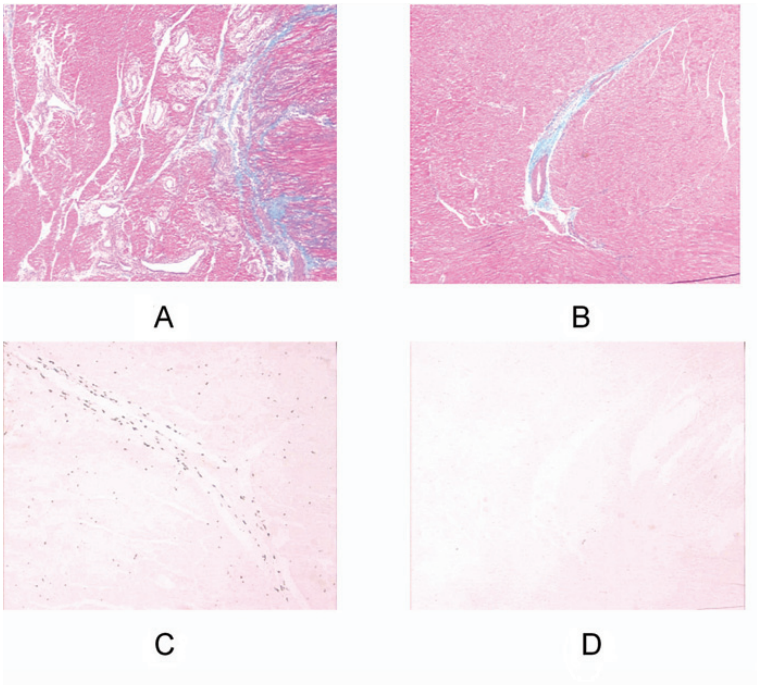

Figure 3. Trichrome staining of ischemic (A) and nonischemic zones (B) in VSTENT-treated animals. New small vessels and capillaries are seen in ischemic zones after VSTENT placement. Bromodeoxyuridine staining for new capillaries and small vessels are similarly seen in ischemic zones (C) versus nonischemic zones (D).

10-fold increase in capillary density in the ischemic region. This level of staining with BrdU implies the creation of new vascular capillary units from pre-existing vessels [Tomanek 1998]. The release of angiogenic factors comes after myocardial injury, ischemia, inflammation, mechanical stress, and myocardial hemorrhage, all of which likely occur with VSTENT placement [Kantor 1999; Simons 2004]. A highly regulated sequence of events, from migration of capillary endothelial cells to lumen formation to finally formation of microvascular networks, typically takes at least 2 weeks to occur with measurable results [Unger 1990; Kohmoto 1996]. This corresponds very closely to our results, which demonstrate a marked increase in perfusion at 3 weeks. Likewise, the patency of the myocardial channel in the VSTENT is also critical to supply the ischemic myocardium in the interval time necessary to increase perfusion. The local milieu conducive to angiogenesis and vascular remodeling may prove to be the most important effect of the VSTENT device.

No adverse side effects related to device placement, hemorrhage, distant thromboses, coronary disruption, or any other technical complications were noted. No inflow or outflow stenoses, kinking, or focal thrombosis was seen. The fear of a local coronary steal syndrome was not found on either microsphere perfusion data or histologic staining, both of which revealed excellent flow to all regions of the myocardium, especially ischemic areas. Device specifications per animal were easily assessed at the time of operation, and any adjustments were readily made. In general, the surgical procedure required much shorter operative times and resulted in less trauma than that seen in other cardiac surgery. Currently, the development of percutaneous methods for VSTENT insertion is underway. As the techniques necessary for implantation are refined, direct LV-to-coronary artery stenting may potentially be applied to all patients requiring myocardial revascularization.

CONCLUSIONS

Direct LV-to-coronary shunts increase myocardial perfusion and can remain patent 3 weeks after the device implantation with standard anti-platelet therapy. Based on preliminary histological data, angiogenesis may be involved and further study investigating mechanisms should be performed.

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