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Effects of Occlusion Devices for Minimally Invasive Coronary Artery Bypass Surgery on Coronary Endothelial Function of Atherosclerotic Arteries

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Louis P. Perrault, MD, PhD, Nathalie Desjardins, BSc, Caroline Nickner, MSc, Pascale Geoffroy, MSc, Jean-François Tanguay, MD, Michel Carrier, MD

Research Center, Department of Surgery and Cardiology, Montreal Heart Institute, Montreal, Quebec, Canada

ABSTRACT

Background: During minimally invasive coronary artery bypass grafting surgery, technical devices are required to obtain an optimal operative field. However, these devices can cause lesions to the endothelium. Previous studies of the effect of devices on coronary endothelial function have been performed on normal coronary arteries. Balloon denudation is followed by regeneration of the endothelium, which is dysfunctional and by intimal hyperplasia resembling atherosclerosis. The Anastaflo™ (Baxter, Mississauga, Ontario, Canada) shunt is a surgical device used during the performance of coronary anastomoses that allows a continuous flow inside the artery during the surgery.

Methods: We compared the effects of three commonly used techniques on endothelial function: 1) using the Anastaflo™ shunt, 2) using Retract-o-tape® (Genzyme, Cambridge, MA) silicone air cushion snaring, and 3) using a bulldog clamp on arteries submitted to balloon denudation 30 days prior to the surgical experiment on an in vivo model of beating heart off-pump coronary artery bypass (OPCAB). Balloon denudation was performed on the left anterior descending artery and on the right coronary artery.The devices were applied for 15 minutes on porcine epicardial coronary arteries, on the beating heart, after median sternotomy. Denuded control rings were taken at the site of denudation on which no devices were applied while non-denuded control rings were taken from the left circumflex artery. The endothelial function of control and instrumented arterial rings was studied in organ chambers filled with a modified Krebs-Ringer bicarbonate solution. After contraction to prostaglandin $F_{2\alpha}$, endothelium-dependent relaxation to serotonin (an agonist coupled to Gi-proteins) and bradykinin (a non–Gi-protein coupled agonist) were compared in the shunting group, the snaring group, the clamping group, and in controls.

Results: There was a significant decrease in the relaxations to serotonin and bradykinin in the denuded control group compared with the non-denuded control group, confirming that balloon denudation caused an endothelial dysfunction. However, there was no significant difference between the denuded control group and the hemostatic device groups (shunting, snaring and clamping) in the relaxation to serotonin.

Conclusion: These results suggest that hemostatic devices used for OPCAB do not cause any greater injury in atherosclerotic coronary arteries already harbouring an endothelial dysfunction. The effects on platelet aggregation, intimal dissection, and plaque rupture remain to be determined.

INTRODUCTION

Minimally invasive coronary artery bypass grafting (CABG) surgery is gaining in popularity and applicability [Acuff 1996] and its advantages are becoming better defined. Coronary bypass surgery performed on beating hearts may allow patients a quicker recovery because anesthetic techniques that are used are significantly different [Maslow 1999]. However, many technical difficulties are encountered in beating-heart surgery: the decreased stability created by the movement of the heart and the presence of blood circulation in the coronary arteries are two of the most important factors. To overcome these problems, new surgical devices and techniques have been developed to

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Address correspondence and reprint requests to: Louis P. Perrault, MD, PhD, Research Center and Surgery Department, Montreal Heart Institute, 5000 Belanger East Street, Montreal, Quebec, H1T 1C8, Phone: (514) 376-3330 (ext. 3471), Fax: (514) 376-1355, Email: lpperrau@icm.umontreal.ca

improve the feasibility of beating-heart surgeries. Stabilizers [Shennib 1997, Maslow 1999, Nicker 2000], shunts, bulldog clamps [Perrault 1996], and epicardial sutures are devices that have a common purpose: to create hemostasis of the operative field while creating a reduction in the range of heart motion. Off-pump coronary artery bypass (OPCAB) surgery allows the performance of CABG without the use of cardiopulmonary bypass and cardioplegia, potentially decreasing total body inflammation, reducing myocardial ischemia, and producing less end-organ damage such as renal failure and stroke.

The Anastaflo™ shunt (Baxter, Mississauga, Ontario, Canada) is a surgical device that allows the performance of coronary anastomosis while maintaining blood flow inside the coronary artery and achieving hemostasis at the operative site. Manipulation and friction of the endothelial wall of the coronary arteries can easily create an endothelial dysfunction by rubbing away endothelial cells. Balloon denudation by angioplasty induces regeneration of endothelial cells that are dysfunctional, 28 days after the procedure, affecting endothelium-dependent relaxations [Borg-Capra 1997] and causes intimal hyperplasia resembling atherosclerosis. Previous studies of the effect of devices on coronary endothelial function have been performed on normal coronary arteries. Because most patients who come to percutaneous or surgical revascularization harbour some degree of systemic and coronary artery endothelial dysfunction associated with the atherosclerotic process, studying the behavior of diseased vessels is paramount and clinically relevant. This study was designed to evaluate the effects of different hemostatic devices used for OPCAB on the endothelial function of atherosclerotic porcine coronary arteries.

MATERIALS AND METHODS

Surgical Technique

All experiments were performed using Landrace swines of both sexes, aged 8 ± 1 weeks with a mean weight of 23.2 ± 1.7 kg. Animals were treated in compliance with the recommendations of the Guidelines on the Care and Use of Laboratory Animals issued by the Canadian Council on Animals, and the Guidelines of the Animal Care and were approved by a local committee.

Coronary Balloon Denudation

The animals were given 650 mg aspirin (ASA) and 30 mg nifedipine 24 hours before the procedure. Following an overnight fast, the animals were sedated by intramuscular injection of 6 mg/kg Telazol (Fort Dodge Laboratories, Fort Dodge, IA) and 50 µg/kg atropine (Abbott Laboratories, Montreal,Quebec, Canada), intubated, and mechanically ventilated with a mixture of oxygen/air. Anesthesia was maintained with 1%–2% isoflurane (Abbott Laboratories, Montreal, Quebec, Canada). An intravenous saline perfusion at a rate of 10 mL/kg/hr was maintained during the procedure. The hematological parameters of each animal were determined, and the electrocardiogram and arterial blood pressure were monitored. Before the procedure, 100 mg xylocaine (Bioniche, Inc., Mississauga, Ontario, Canada) and 150 U/kg heparin (Leo Pharma, Inc., Ajax, Ontario, Canada) were given to maintain the activated clotting time over 300 sec.

Percutaneous Transluminal Coronary Angioplasty (PTCA) Procedure

After insertion of an 8-Fr arterial sheath in the femoral artery, an 8-Fr guiding catheter was advanced into the ascending aorta and placed in each coronary ostium. After administration of 100 mg intracoronary nitroglycerin (Sabex, Boucherville, Quebec, Canada), a baseline coronary angiogram was performed using a contrast solution (MD76; Mallinckrodt Medical, Pointe-Claire, Quebec, Canada). Quantitative coronary artery measurements were obtained using the on-line View system (view 1.2.3, Electromed International, St-Eustache, Quebec, Canada). Baseline minimal lumen and reference diameters of each coronary artery were determined using the guiding catheter as a reference diameter.

A 40-mm length noncompliant angioplasty balloon catheter was used for the procedure. The balloon size was selected to obtain a balloon to artery ratio of 1.1:1.3. An 8-Fr guiding catheter was advanced over a 0.035-in guidewire into the ascending aorta and the left or right coronary ostium was engaged after removal of the guidewire. The balloon catheter was advanced over a 0.014-in guidewire. PTCA was performed in two coronary arteries. A maximum inflation pressure of 10–14 atmospheres for 30 seconds was used twice. After deflation and retraction of the angioplasty balloon, 100–200 µg of intracoronary nitroglycerin was given again and an angiogram was performed. Quantitative coronary digital measurements of the inflated balloon, reference vessel diameter, and PTCA segments were obtained. At the end of the procedure, the femoral sheath was removed and swines were allowed to recover and were returned to animal care facilities.

In Vivo OPCAB Model

Open heart surgery was done electively on the swines 30 days after balloon denudation. After anesthesia with an intramuscular-mixture injection of ketamine (20 mg/kg; Rogarsetic, Quebec City, Canada) and xylazine (2 mg/kg; Rompun, Ontario, Canada), swines were artificially ventilated with an oxygen/air mixture (3:2) and light anesthesia was supported by Halothane 1% (Halocarbon Laboratories, Markham, Ontario, Canada). Hair was shaved at the operative field (chest and neck) and the skin was disinfected for surgery. Venous access was obtained through the left jugular vein for volume replacement with Ringer's lactate, and arterial cannulation was performed through the left internal carotid artery for blood pressure monitoring. Heparin was given at a concentration of 300 units/kg of body weight.

After median sternotomy and pericardial suspension, the segments of the left anterior descending and right coronary arteries (LAD and RCA) were dissected at the sites of balloon denudation, done 30 days prior to the operation, and prepared for shunt insertion. Proximal snaring was achieved using Retract-o-tape® (Genzyme, Cambridge, MA) silicone air cushion, and bulldog clamps were used for distal clamping. The shunt was inserted into the lumen after a 5-mm longitudinal arteriotomy and was left in place for 15 minutes, as were the other two devices. The arteries were reperfused for 30 minutes after application of the devices.

Vascular Reactivity

After application of the devices and reperfusion, the heart was rapidly excised and placed in modified Krebs-bicarbonate solution (composition in mmol/L: NaCl 118.3, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, glucose 11.1, $CaCl_2$ 2.5, $NaHCO_3$ 25, and ethylenediaminotetraacetic acid 0.026). Oxygenation was ensured using a carbogen mixture (95% O_2 and 5% CO_2). The two instrumented arteries, LAD and RCA, were dissected free of the myocardium and epicardial tissue and were divided into rings, 4 mm in width. Markers were kept in place during the coronary dissection to identify the location of the sites of device application. The instrumented segments were divided into sections: the snaring, clamping, shunting and denuded control sites. The left circumflex artery was also dissected and used as a non-denuded control.

The endothelial function of control rings and artery rings subjected to device application was studied in organ chambers filled with modified Krebs-bicarbonate solution (20 mL at 27°C , see above for composition) and oxygenated with the carbogen mixture. The rings were suspended between two metal stirrups, one of which was connected to an isometric force transducer. Data were collected with a data acquisition software (IOS3; Emka Inc, Paris, France). Response to 5-hydroxytryptamine creatine sulfate (serotonin) and bradykinin were compared.

Each preparation was stretched to its active length curve (usually 3.5 g), as determined by measuring the contraction to potassium chloride (30 mmol/L) at different levels of stretch, and then stabilized for 30 minutes. The maximal contraction was determined with potassium chloride (60 mmol/L) and rings were excluded if they failed to contract to potassium chloride (exclusion rate of less than 5%); baths were then washed. All studies were performed in the presence of indomethacin (10^{-5} mol/L, to exclude the production of endogenous prostanoids), propanolol (10^{-7} mol/L, to prevent the activation of β-adrenergic receptors), and ketanserin (10^{-6} mol/L, incubated 45 minutes before the addition of serotonin to block serotonin 5HT-2 receptors).

After 45 minutes of stabilization, prostaglandin $F_{2\alpha}$ (range 2×10^{-6} to 3×10^{-5} mol/L) was added to achieve a contraction averaging 50% of the maximal contraction to KCL (60 mmol/L). Endothelium-dependent relaxations were studied using serotonin (10^{-10} to 10^{-5} mol/L; an agonist binding to Gi-protein receptors coupled to $5HT_{1D}$), and bradykinin (10^{-12} to 10^{-6} mol/L; an agonist Gq-protein–dependent). Endothelium-independent relaxations were studied using a bolus of sodium nitroprusside (SNP, 10^{-5} mol/L) at the end of the experiment.

Histology

At the end of organ chamber experiments, the coronary rings were fixed in 10% formaldehyde for 20 minutes at their optimal tension. All formalin-fixed arteries were embedded in parafin and 5-micron sections were stained with movat pentachrome. Each section was examined for the presence, extent, and distribution of intimal thickening, luminal narrowing, and disruption of the internal elastic lamina by light microscopy. A quantitative ratio of intimal hyperplasia to the media was then calculated to obtain the extent of intimal thickening.

Drugs

All solutions were prepared daily. Bradykinin, 5-hydroxytryptamine creatine sulfate (serotonin), indomethacin, ketanserin, and sodium nitroprusside were purchased from Sigma Chemical Co. (Ontario, Canada). Propanolol was purchased from Biomol Research Laboratories, Inc. (Plymouth Metting, PA) and prostaglandin $F_{2\alpha}$ was purchased from Cayman Chemical Company (Ann Arbor, MI).

Statistical Analysis

Contractions to $PGF_{2\alpha}$ are expressed as a percentage of the maximal contraction to potassium chloride (60 mmol/L) for each group and expressed as mean ± standard error of the mean; *n* refers to the number of animals studied. Relaxations are expressed as a percentage of the maximal contraction to $PGF_{2\alpha}$ for each ring. Analysis of variance with repeated measures studies were performed to compare dose-response curves. Differences were considered to be statistically significant when p was less than 0.05.

RESULTS

Vascular Reactivity

Contraction: There were no statistical differences in the amplitude of contraction to prostaglandin $F_{2\alpha}$ (range 2×10^{-6} to 3×10^{-5} mol/L) in all groups compared with controls (data not shown).

Endothelium-Dependent Relaxations: There was a statistically significant decrease of relaxations to serotonin in all rings compared with the non-denuded control. There were no statistical differences in the device application groups (shunt, snare, and bulldog clamp) compared with the denuded control rings (Figure 1,).

There was a statistically significant decrease of relaxations to bradykinin in all groups compared with the non-denuded control. There was no statistical difference in relaxation in the shunting group compared with the denuded control group (Figure 2,).

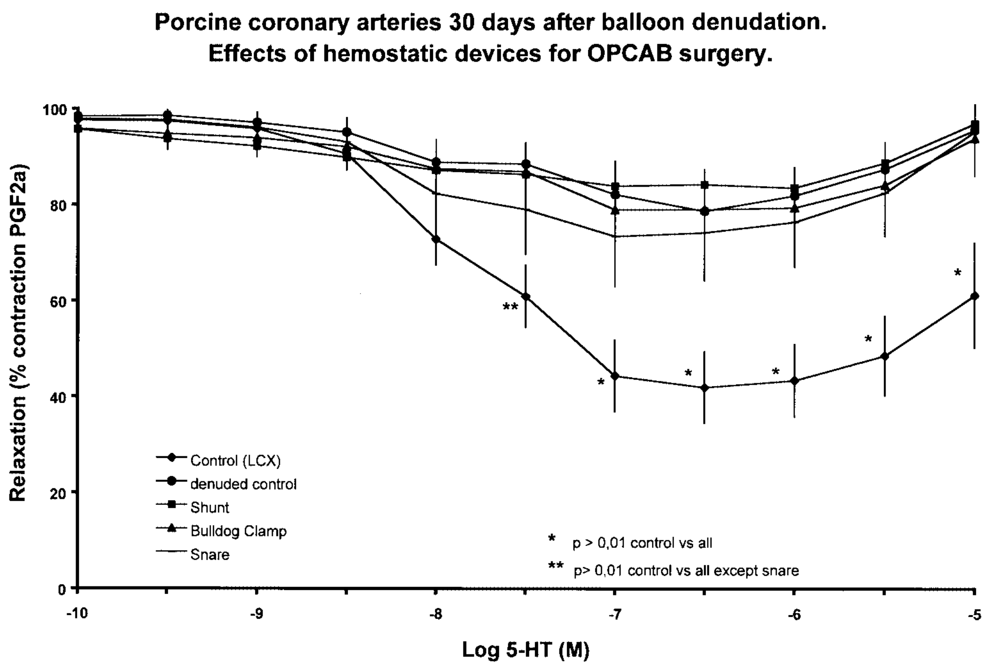

Figure 1. Cumulative concentration-relaxation curves to serotonin in rings of porcine left anterior descending and right coronary arteries with endothelium subjected to angioplasty 30 days prior to being subjected to coronary shunting, snaring, or clamping.

Intimal Thickening: There was an increase in the ratio of intimal thickening to media in the denuded arterial rings (LAD and RCA) as shown by insertion of the intima into the artery lumen (Figure 3, ◎).

DISCUSSION

OPCAB is increasingly performed in many centers around the world [Dagenais 1999, Cartier 2000]. Potential advantages

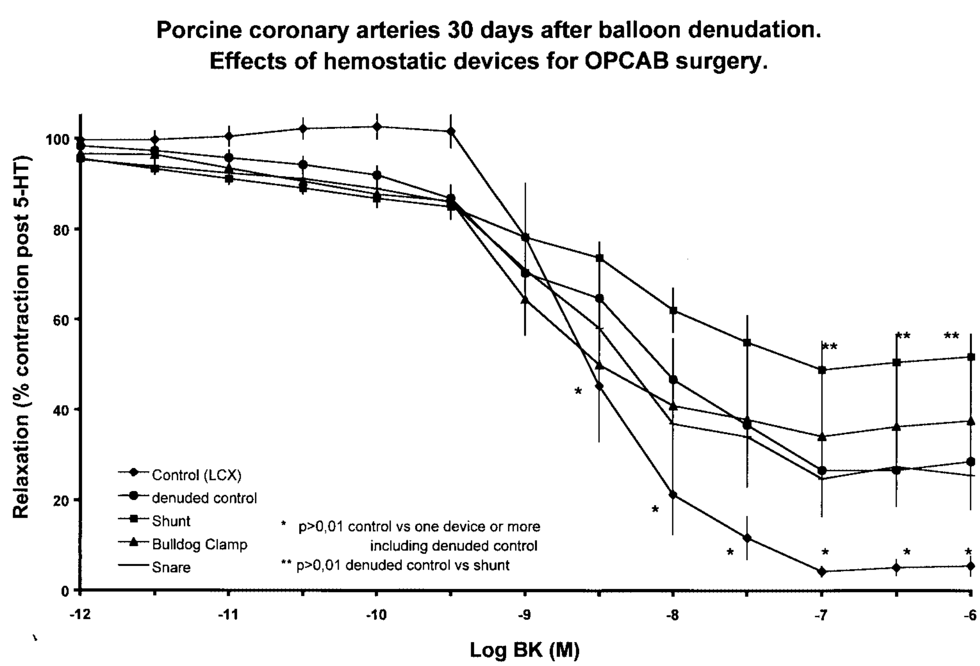

Figure 2. Cumulative concentration-relaxation curves to bradykinin in rings of porcine left anterior descending and right coronary arteries with endothelium subjected to angioplasty 30 days prior to being subjected to coronary shunting, snaring, or clamping.

Figure 3. Representative photographs of coronary arteries subjected to angioplasty 30 days prior to the in vivo manipulations showing insertion of the intima into the arterial lumen.

include minimization of myocardial ischemia–reperfusion injury and avoidance of the use of cardiopulmonary bypass and its potential negative effects. However, alternative techniques to stabilize the heart and obtain a clear operative field must be used in order to perform high-quality anastomoses as when done with cardiopulmonary bypass and cardiac arrest.

Endothelial cells are at the center of the homeostasis of the vascular wall but are fragile to manipulations as shown by the simple way of denuding a segment of coronary artery by gentle rubbing of a wooden stick into the luminal surface of an arterial segment [Perrault 1996]. Denudation of the endothelial-cell coverage results in a decrease of endothelium-dependent relaxations [Fonger 1995] and a propensity for spasm and endothelium dysfunction after regeneration. Application of the Anastaflo™ shunt (Baxter, Mississauga, Ontario, Canada) causes an endothelial denudation and dysfunctional Gi-protein–mediated relaxations [Chavanon 1999] associated with insertion and removal of the shunt in normal coronary arteries.

Patients who undergo OPCAB or minimally invasive direct coronary artery bypass usually do so for advanced coronary atherosclerosis. There is also a 50% rate of progression of atherosclerosis in the native coronary arteries after a CABG [Bourassa 1984]. Coronary artery bypass is therefore usually performed on dysfunctional arteries, whether it is a first bypass or a reoperation. Balloon denudation 30 days prior to the in vivo manipulations induced the formation of intimal hyperplasia resembling atherosclerosis, creating a model of atherosclerotic arteries on which the hemostatic devices were applied.

Previous studies from our laboratory have shown that application of the Anastaflo™ shunt (Baxter, Mississauga, Ontario, Canada) on normal porcine coronary arteries creates a decrease of endothelium-dependent relaxations compatible with endothelial dysfunction. In the present study, denuded arteries showed a generalised dysfunction affecting both Gi- and Gq-protein–mediated relaxations. Application of hemostatic devices (the snare, the bulldog clamp, and the Anastaflo™ shunt) on atherosclerotic arteries did not cause a significantly greater dysfunction of Gi-protein endothelium-dependent relaxations when applied to the atherosclerotic arteries. The alteration also involved endothelium-dependent Gq-protein–mediated relaxations as shown by the decreased relaxations to bradykinin in the device groups compared with the control group. The contractile function and endothelium-independent relaxations were unaffected by the use of all techniques, demonstrating the integrity of the underlying smooth muscle cells.

The Anastaflo™ shunt (Baxter, Mississauga, Ontario, Canada) cannot be considered an atraumatic device since it induces the loss of endothelial cell coverage in normal porcine coronary arteries. However, in the clinical field of surgical myocardial revascularization, the use of the Anastaflo™ shunt for the performance of coronary artery bypass is predominantly done on atherosclerotic arteries. Under those conditions, use of a shunt as a hemostatic device may be considered safe because it does not create a greater dysfunction than the one already present in atherosclerotic coronary arteries that are already harbouring an endothelial dysfunction.

Because it has been shown that hemostatic devices may also cause endothelial dysfunction at the site of application when used on disease-free porcine coronary arteries [Perrault 2000], these devices should be used with caution on healthy or minimally-diseased arteries. Great care should also be taken not to injure segments of the target coronary artery distal to the anastomosis because those segments may not be presenting atherosclerotic lesions prior to the bypass. The effects of hemostatic devices on platelet aggregation, intimal dissection, and plaque rupture in OPCAB remain a concern [Hangler 2000] and mandate further studies.

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