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Abstract

Introduction: Repetitive, brief coronary artery occlusions produce collateral development in experimental animals. This model causes coronary collateralization in a highly reproducible fashion, but the process is very labor intensive. We report the design and use of a fully automated hydraulic coronary occlusion device capable of producing repetitive coronary occlusions and enhancement of coronary collateral development in dogs. **Methods**: The device consists of analog electronics that allow adjustment of occlusion number, frequency, pressure and duration, and mechanical components responsible for the coronary occlusion. The motor and piston of the device are coupled to a chronically implanted hydraulic vascular occluder placed around the left anterior descending coronary artery (LAD) of dogs instrumented for measurement of systemic and coronary hemodynamics. One group of dogs (*n*=6) underwent brief (2 min) LAD occlusions once per hour, eight times per day, 5 days/week for 3 weeks to stimulate collateral development (measured using radioactive microspheres). Another group of dogs (*n*=6) that did not receive repetitive occlusions served as controls. **Results**: The device reproducibly produced repetitive LAD occlusions for the duration, frequency, and time interval initially programmed. A time-dependent increase in transmural collateral blood flow was observed in dogs undergoing repetitive occlusions using the device. Collateral blood flow was unchanged in dogs that did not undergo occlusions. **Discussion**: The automated occluder device reliably produces repetitive coronary occlusions and may facilitate further study of coronary collateral development in response to chronic myocardial ischemia.

Keywords

Angiogenesis, Automated coronary occluder device, Coronary collateral circulation, Dog, Methods, Myocardial ischemia

1. Introduction

Development of coronary collateral blood vessels occurs in response to chronic myocardial ischemia. Coronary collaterals are important alternative sources of blood flow to ischemic myocardium that improve contractile function and reduce injury to subsequent ischemic events. The growth of new collateral vessels (termed “angiogenesis”) and the enlargement of preexisting collaterals occur as a result of the concerted actions of several angiogenic mitogens that are released during ischemia Banai et al., 1994, Lazarous et al., 1996, Matsunaga et al., 2000, Matsunaga et al., 2002, Schaper, 1993, Sharma & Schaper, 1993, Tessmer et al.,

2002, Weihrauch et al., 1998. Repetitive episodes of brief coronary artery occlusion and reperfusion have been shown to produce coronary collateral development in a variety of animal species Kersten et al., 1997, Kersten et al., 1999, Kersten et al., 1995 and may also have a pathophysiological basis in humans with coronary artery disease (Fujita et al., 1988). This repetitive coronary occlusion model produces collateralization in a highly reproducible fashion, but the process is very labor intensive because brief (typically between 1 and 3 min duration) coronary occlusions interspersed with reperfusions must be performed at regular intervals (15–120 min) several times per day (4–24) for several weeks (3–12) depending on the animal species used and the degree of collateral development desired (Kersten et al., 1999).

We report the design, construction, and use of an automated hydraulic coronary artery occlusion device capable of temporally producing the repetitive brief coronary occlusions of different durations and occurring at different intervals required to reliably develop coronary collaterals in dogs. The two-part system consists of an electric control interface coupled to mechanical components that drive and retract a piston responsible for hydraulic occlusion and reperfusion of a canine coronary artery in vivo. The device was rigorously constructed of very durable components to withstand the typical activities of conscious, chronically instrumented dogs over a span of several weeks of continuous use. We used this automated coronary occlusion device to verify our previous results Kersten et al., 1995, Kersten et al., 1997, indicating that repetitive, brief (2 min) coronary occlusions conducted eight times per day, 5 days/week for 3 weeks stimulate the development of the coronary collateral circulation. We have previously demonstrated that this model of repetitive coronary occlusion produces collateral development in dogs as indicated by time-dependent increases in collateral blood flow, reductions in reactive hyperemic responses, and improvements in regional contractile function during acute coronary artery occlusion Kersten et al., 1995, Kersten et al., 1997.

2. Methods

2.1. Use and care of animals

All experimental procedures and protocols used in this investigation were reviewed and approved by the Animal Care and Use Committee of 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 were in accordance with the “Guide for the Care and Use of Laboratory Animals” of the National Institutes of Health (National Academy Press, Washington, DC, 1996).

2.2. General device design and specifications

The device consists of two major elements constructed of commercially available parts: the user interface and its associated analog electronics and the mechanical components responsible for coronary artery occlusion (Fig. 1). These two main components are housed in separate heavy-gauge aluminum boxes that are joined by a circular conducting connection cable. A schematic diagram detailing the electronic design of the device is illustrated in Fig. 2. For simplicity, a functional schematic has also been depicted (Fig. 3). The user interface contains two circuit boards manufactured in our laboratory. The top circuit board contains the front panel components (i.e., user controls) including an error fault LED and a digital occlusion counter display covered with a protective membrane. The bottom circuit board contains the main logic circuitry that consists of discrete devices and CMOS integrated circuits powered by four AA batteries arranged in parallel to yield ∼6 V. The average quiescent power drain is 6 mA, with current bursts approaching 100 mA during motor movement. The device is capable of imparting varying hydraulic pressures that are more than sufficient to occlude canine coronary arteries in vivo.

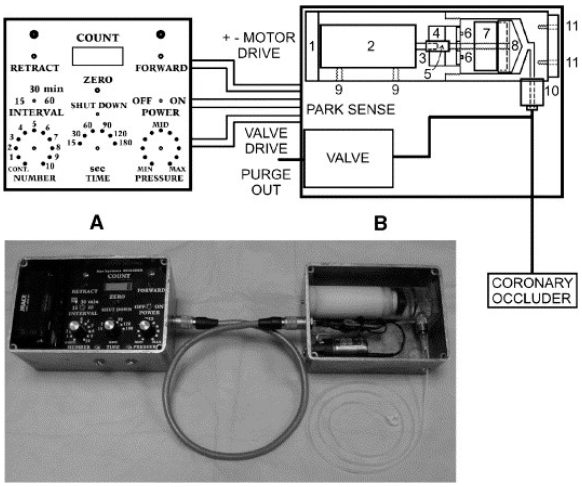

Fig. 1. Schematic illustrations (top panels) and corresponding photographs (bottom panels) depicting the electrical (A) and the mechanical (B) components of the automatic coronary occlusion device. (1–3) Motor end cap, body, and shaft. (4 and 5) Shaft bearing and coupler. (6) Park-sense switch. (7 and 8) Piston with rubber syringe tip. (9) Motor lock screws. (10) Luer-lock fitting with rubber gasket. (11) Side mount screws.

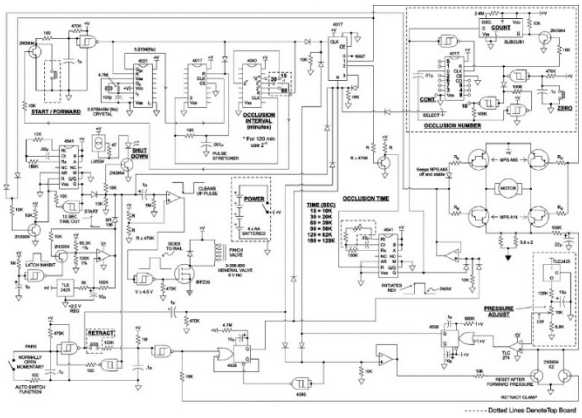

Fig. 2. Detailed schematic illustrating the electronic design of the automated hydraulic coronary occlusion device.

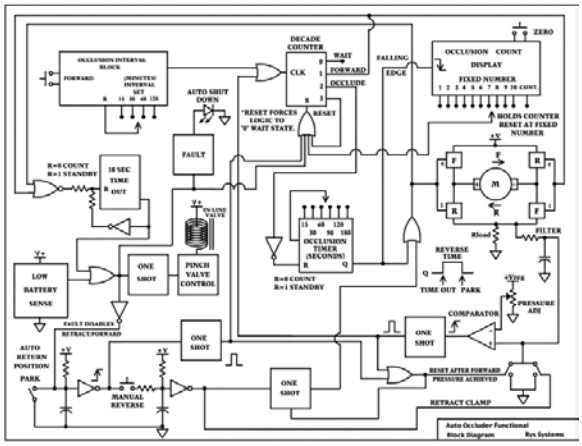

Fig. 3. Functional schematic illustrating operational control and features of the automated hydraulic coronary occlusion device.

2.3. User control interface

The front panel of the user control interface contains a power switch (TT11AGPC1; Augat, Mansfield, MA), subcub six-digit component counter display (SubCub 1000; Red Lion Controls, York, PA), and forward, retract, and zero buttons (39-424; Grayhill, LaGrange, IL). An adjustable potentiometer controls the occlusion pressure (381N-10K; Clarostat, Richardson, TX). Switches allow for variation in the duration (56DP30-01-2-AJN; Grayhill), number (56DP30-01-1-AJN; Grayhill), and time interval (TT21PAGPC1; Augat) between occlusions. The occlusion durations ranging between 15 and 180 s may be preset using the control panel. The number of occlusions performed each day may be fixed between 1 and 10 or may be set to a "continuous" mode that allows for a virtually unlimited number of daily occlusions that cease only as a result of power failure or mechanical malfunction. The time duration between each occlusion may also be preset to 15, 30, or 60 min (Fig. 1).

2.4. Mechanical occluder components

A Delrin cylinder is attached to an aluminum box using heat-annealed Plexiglas end caps (Fig. 1B). Delrin and heat-annealed Plexiglas were specifically chosen for long-term durability and superior machining characteristics. The cylinder aligns the park-sense circuit and houses the motor shaft and body (1516E002SLT 115/2 900:1 K185; Micro Mo, Clearwater, FL), the shaft bearing and coupler, and the main piston. The piston is designed to accept the rubber tip from a standard 20-ml syringe (Becton-Dickinson, Franklin Lakes, NJ) and contains 4 ml of distilled water. A luer-locked fitting with a rubber gasket to prevent leakage is attached to the Delrin cylinder and accepts nondistensible tubing that is attached to a relief valve (3-206-900; General Valve, Fairfield, NJ) and the chronically implanted coronary artery occluder (see below).

2.5. Device operation

The temporal function of the device is determined by the status of the decade counter, assuming the total supply voltage is sufficient to power the device. After powering "on," the device is inoperable for a 10-s circuit initialization period. The device then enters a "default standby" mode as indicated by the "wait" state in Fig. 3. An occlusion cycle is generated after the occlusion interval is timed out or by manual depression of the forward button on the control interface after device initiation. In the "forward" mode (logic "0"), a complementary pair of transistors (shown as blocks in Fig. 3) is biased to produce forward movement of the motor. This motor movement creates torque against the combined load of the piston, cylinder, and coronary occluder and simultaneously imparts a voltage on the comparator input (filter out). Motor movement is discontinued when this voltage exceeds the preset voltage of the pressure potentiometer on the user interface. The minimum pressure setting required to adequately occlude the coronary artery is determined by the user as the pressure associated with complete cessation phasic coronary blood flow velocity. The device must achieve the desired pressure within 10 s or it will enter a "shutdown" operational mode. Once the desired pressure has been reached, the comparator out drives a one shot that sequences the decade counter to an occlude mode, resets the previous value of the filter out, and disables the 10-s shutdown counter. When the occlusion has been completed, the timer enters a logic "1" that provides the drive for a complementary pair of transistors associated with the "reverse" mode of motor operation and also reactivates the 10-s shutdown timer. The piston then returns to the original position from which only forward motor movements are subsequently possible. The decade counter is then reset, and the device enters a "wait" state of operation. The occlusion count display also indicates that a successful occlusion was completed.

2.6. Automatic shutdown

The device was designed with an automatic shutdown feature to prevent permanent coronary occlusion due to malfunction. Several possible events during routine operation may cause the device to automatically shutdown, resulting in the illumination of the fault LED on the control panel. For example, a low battery condition occurs when the supply voltage falls below 4.8 V and results in automatic shutdown. Device shutdown will also occur if

the duration of forward or reverse piston movements exceeds 10 s. The forward and reverse piston movements and the decade counter function are disabled when a fault condition is detected. Importantly, an inline valve relieves hydraulic pressure in the closed coronary occluder pathway during any fault condition. This safety feature prevents inadvertent prolonged occlusion of the coronary artery from occurring.

2.7. Surgical instrumentation

The implantation of instruments has been previously described in detail Kersten et al., 1995, Kersten et al., 1997. Briefly, conditioned mongrel dogs (*n*=12) of either sex weighing between 25 and 30 kg were fasted overnight and anesthetized with intravenous propofol (8 mg/kg). After endotracheal intubation, anesthesia was maintained with isoflurane (inspired concentration between 1.5% and 2.0%) in 100% O_2 using positive pressure ventilation. Fluid deficits were replaced with 500-ml 0.9% saline, which was continued at a rate of 3 ml/kg/h for the duration of the surgery. Acid–base status and arterial blood gas tensions were maintained within the normal range by adjustment of respiratory rate and tidal volume. Temperature was maintained with a heating blanket. Under sterile operating conditions, a thoracotomy was performed in the left fifth intercostal space. The phrenic nerve was identified and protected. The pericardium was opened and the heart was temporarily suspended in a pericardial cradle. Heparin-filled catheters were positioned in the right atrial appendage and thoracic aorta for administration of fluid and measurement of arterial blood pressure, respectively. A heparin-filled catheter was also placed in the left atrial appendage for the administration of radioactive microspheres. A miniature hydraulic occluder (In Vivo Metric, Healdsberg, CA) was positioned around the left anterior descending coronary artery (LAD) for the production of coronary artery occlusion and reperfusion. A precalibrated Doppler flow probe was positioned around the LAD immediately distal to the hydraulic occluder for measurement of phasic coronary artery blood flow velocity. All instrumentation was secured, tunneled between the scapulae, and exteriorized via several small incisions. The pericardium was left open, the chest wall was closed in layers, and the pneumothorax was evacuated by a chest tube. Each dog was fitted with a jacket (Alice King Chatham, Los Angeles, CA) to prevent damage to the instruments, the catheters, and the hydraulic occlusion device that were contained in aluminum boxes within the jacket pockets (Fig. 4). All dogs received epidural morphine (0.1 mg/kg) for postoperative analgesia. Antibiotic prophylaxis consisted of cephalothin (1 g) and gentamicin (4.5 mg/kg). Anesthesia was discontinued and emergence was allowed to occur. Dogs were allowed to recover for 7 days before subsequent experimentation.

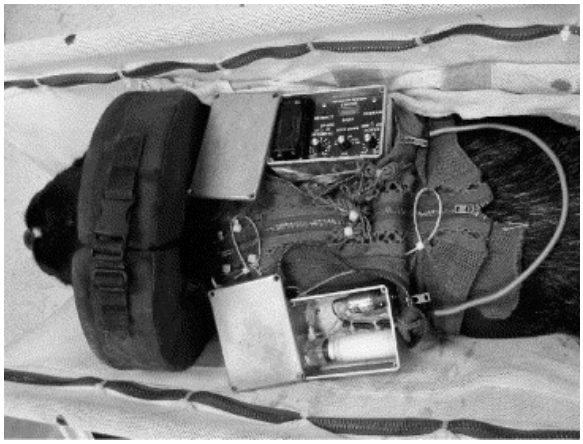

Fig. 4. Photograph depicting the aluminum boxes containing the electrical and mechanical components of the automated hydraulic coronary occlusion device stored in the jacket pockets of a typical conscious, chronically instrumented dog.

2.8. Experimental protocol

Systemic and coronary hemodynamics were monitored daily, recorded on a polygraph (Model 7758A; Hewlett-Packard, San Francisco, CA), and digitized by a computer interfaced with an analog-to-digital converter. One group of dogs (*n*=6) was assigned to receive 2-min LAD occlusions at hourly intervals eight times per day, 5 days/week for 3 weeks using the automated occlusion device. We have previously demonstrated that this schedule of repetitive brief coronary occlusions produces an extensive coronary collateral circulation in this canine model Kersten et al., 1995, Kersten et al., 1997. Hemodynamics was monitored before, during, and after each occlusion. A second group of dogs (*n*=6) was instrumented identically but did not undergo repetitive coronary occlusions (sham). Transmural coronary collateral blood flow was assessed using the radioactive microsphere technique at 1, 7, 14, and 21 days of repetitive LAD occlusions. At the completion of the experiment, each dog was euthanized with an overdose of sodium pentobarbital, and the position of implanted instruments was confirmed.

2.9. Measurement of regional myocardial perfusion

Microspheres (15±2 μm, mean±S.D.) labeled with ^{95}Nb, ^{141}Ce, and ^{103}Ru were used to measure myocardial perfusion as previously described Domenech et al., 1969, Kersten et al., 1995, Warltier et al., 1981. Briefly, microspheres were administered into the left atrium as a bolus. A few seconds before injection, a timed collection of reference arterial blood flow was started from the aortic catheter at a rate of 7 ml/min for 3 min. Transmural tissue samples were selected from the ischemic region and subdivided into subepicardial, midmyocardial, and subendocardial layers of approximately equal thickness. Samples were weighed and the activity of each isotope was determined. Similarly, the activity of each isotope in the reference blood flow sample was assessed. Tissue blood flow (ml/min/g) was calculated as $Q_r C_m / C_r$, where Q_r is the rate of withdrawal of the reference blood flow sample (ml/min), C_m is the activity (cpm/g) of the myocardial tissue sample, and C_r is the activity of the reference blood flow sample. Transmural blood flow was considered to be the average of the subepicardial, midmyocardial, and subendocardial blood flows.

2.10. Statistical analysis

Statistical analysis of data between groups was performed using analysis of variance (ANOVA) with repeated measures followed by Student–Newman–Keuls' test. A probability (*P*) value less than .05 was considered statistically significant. All data are expressed as means±S.E.M.

3. Results

All dogs survived the surgical instrumentation without complication. The postoperative course of each dog was also uncomplicated. No failures or malfunction of the automated occlusion device were observed during the course of experimentation. Each occlusion produced by the device completely abolished LAD blood flow velocity for the duration, frequency, and time interval initially programmed (Fig. 5). No difficulties were observed in the adjustment of minimal pressure required to obtain LAD occlusion. Only slight adjustments in this minimal occlusion pressure were required over the 3-week duration of each experiment. A time-dependent increase in transmural coronary collateral blood flow was observed in dogs undergoing repetitive LAD occlusions using the automated coronary occlusion device (Fig. 6). In contrast, transmural collateral blood flow was unchanged in dogs that were not exposed to repetitive occlusions.

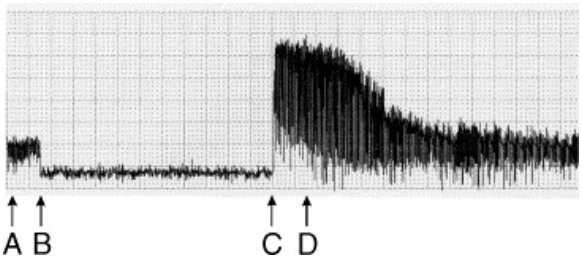

Fig. 5. Changes in phasic LAD coronary artery blood flow velocity in response to activation of the automated occlusion system. Representative chart recording illustrating blood flow velocity under resting conditions (A), at the initiation (B), and at the completion (C) of a coronary artery occlusion and at the peak reactive hyperemic response during reperfusion (D).

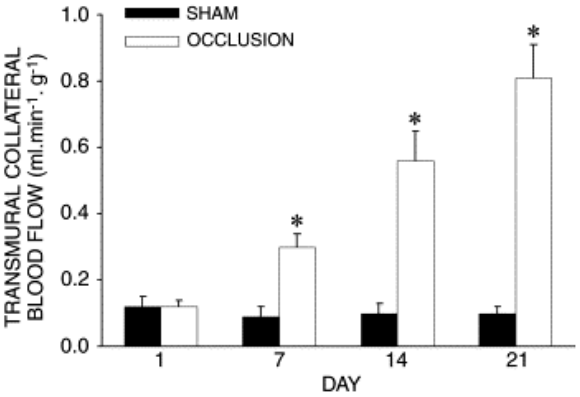

Fig. 6. Temporal increases in transmural coronary collateral blood flow in the ischemic (LAD) region in dogs undergoing repetitive brief coronary occlusions using the automated device on experimental days 1, 7, 14, and 21. *P<.05, significantly different from sham.

4. Discussion

In the current investigation, we report the design, operation, and application of an automated hydraulic occlusion device to reproducibly cause coronary collateralization in conscious dogs. The results indicate that the repetitive brief occlusions produced by the device progressively increase transmural coronary collateral blood flow over a 3-week period to a similar degree as has been observed in our previous studies Kersten et al., 1995, Kersten et al., 1997, Matsunaga et al., 2000, Matsunaga et al., 2002, Weihrauch et al., 1998 using the more laborious manual occlusion method. The current report describes the use of this device in dogs, but the device may also be used in other experimental animal species with an appropriately sized implanted coronary occluder and making adjustments in minimal occlusion pressure on the control interface. The device was technically difficult to design and build, but all of the parts used in its construction are readily available from commercial sources, and a capable engineer should be able to replicate the device using the design information provided in this report. Additional details regarding device fabrication are available from the authors upon request.

Development of the automated coronary occlusion device allows laboratory personnel to enroll multiple dogs in a single protocol limited only by the number of occlusion systems. Researchers are also able to work on additional protocols simultaneously with occasional monitoring of each dog. A previous experiment from our laboratory used the current device to deliver 2-min coronary artery occlusions once each hour, 24 h/day for 7 days. The benefits to automating this process are obvious, as manual occlusion would require multiple shifts of laboratory personnel and also would prevent the dog from sleeping for the duration of the protocol.

Previous studies have reported the design and implementation of devices designed to produce repetitive vascular occlusion. Rubin, Quilter, and Battagin (1978) initially described an integrated circuit-based automatic timer device for the repetitive inflation of limb pneumatic cuffs. More recently, Caldwell et al. (1989) reported the design of an automatic coronary occluder system that shares several similarities with the current device. In particular, the electronics of the present and previous (Caldwell et al., 1989) devices make use of similar timer, decade counter, one shot driver, and comparator designs. However, these elements represent standard approaches in electrical engineering design. Importantly, the previously described device (Caldwell et al., 1989) was not subsequently validated in an established model of coronary collateral development that requires over 100 successful brief coronary occlusions and reperfusions over a span of 3 weeks. In comparison, the reliability of the current device is exceptional as demonstrated by successful performance in ~50 dogs instrumented with the automated occlusion system over a 1-year period. In addition, the current device incorporates many features that increase the longevity, improve the mechanical performance, and enhance the safety of the automated occlusion device. The mechanical and electrical components were strictly separated in individual aluminum boxes to ensure that the electronic circuit boards remained clean and dry during prolonged use. The previously described device (Caldwell et al., 1989) combined the mechanical and electrical units in a single box, and a hydraulic leak in the syringe system may have theoretically damaged the electronic components under these circumstances.

A fail-safe mechanism for battery loss was incorporated into the present design. A low battery state will render the device inactive at any time during its normal operation and allow complete decompression of the hydraulic pressure in the coronary occluder via an inline valve during this fault condition. Automatic motor reversal and subsequent device shutdown will also occur if forward motor movement time exceeds 10 s. These objectives are important because the previous device may have maintained complete mechanical coronary occlusion if a power failure occurred before subsequent motor reversal. Such a situation may lead to the development of an acute myocardial infarction or lethal cardiac arrhythmia. The present occluder design used a damped park switch rather than a rear plunger stop as described in the previously characterized occlusion system (Caldwell et al., 1989). This park switch was concentrically located behind the piston and contained a coil spring load that acted as a shock absorber for the piston. This important design modification alleviates unnecessary wear on the motor and lead screw. The present device employed an unique approach to reset the motor load filter output. When reset, the pressure potentiometer circuit will detect a voltage value without any residual voltage (such as may have been generated by the previous motor cycle) to ensure that the desired occlusion pressure is always achieved. The forward and reverse switches on the user control panel were also debounced to eliminate inadvertent occlusion or reperfusion by the user or those accidentally generated by the dog's movements.

There are a number of potential limitations in the current automated coronary occlusion device design and operation that require additional comment. We have found that the batteries should be routinely replaced every 72 h to ensure an adequate system power. Although device integrity is rarely compromised, several device components require occasional maintenance. The rubber syringe tip mounted on the motor piston head needs to be checked occasionally and may show substantial wear or require replacement after several hundred occlusion-reperfusion cycles. The cable connecting the user control interface to the occlusion device should be checked periodically and replaced as needed because pin corrosion occurs intermittently despite gold plating. Mechanical malfunction of the device after battery replacement is most often associated with excessive motor wear and is consistently resolved by replacing the motor. The hydraulic pressures within the coronary occluder that were generated by the device were not specifically measured in situ in the current investigation. A previous study (Caldwell et al., 1989) demonstrated that hydraulic pressures as great as 1000 mm Hg may be developed in this type of automated device. Instead, we choose to manually adjust the pressure potentiometer in order to obtain a minimum pressure required to occlude the coronary artery. This technique mimics the process of hydraulic occlusion with manual inflation, may act to reduce the risk of coronary vascular damage from

excessive device-induced occlusion pressure, and may limit the possibility of inadvertently rupturing the cuff of the chronically implanted occluder. Repetitive coronary artery occlusions may have an impact on the arterial structure of the LAD coronary artery at the site of the hydraulic occluder. However, classification of histological abnormalities in this localized region has not yet been performed.

In summary, we report the details of a new automated hydraulic coronary occlusion device and describe the use of this device for the induction of coronary collateral growth in conscious, chronically instrumented dogs. The results indicate that the device is safe, allows great flexibility in occlusion programming, and delivers consistent, reliable performance over several weeks of continual use in this setting. The device will facilitate further study of coronary collateral development in response to chronic myocardial ischemia.

Acknowledgments

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