Endothelial Cell Stress with Arterial Re-entry During Bypass

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Abstract

High velocity arterial blood re-entry during cardiopulmonary bypass is known to be associated with numerous potential deleterious effects. One of these may be endothelial cell stress with resulting intimal injury. Previous studies by others would suggest that endothelial cell injury of this type could result in long-term vascular disease. To test the hypothesis that arterial re-entry during cardiopulmonary bypass will induce endothelial injury, six mongrel dogs were divided into three study pairs: control (no bypass), continuous (non-pulsatile) bypass, and pulsatile bypass. Evans blue dye was used as a marker of aortic endothelial cell injury. Following each experimental period, the aorta was removed en bloc and examined for Evans Blue Dye staining. Sections of aortic tissue were also examined histologically for cellular damage.

All subjects tolerated the study period without incident. Gross tissue examination of the control subject aortic segments revealed no Evans Blue Dye staining. Histologic examination of these segments was unremarkable. Gross tissue examination of the bypass subject aortic segments revealed Dye staining in all cases with the pulsatile bypass pair staining more extensively and heavily than the continuous bypass pair. Histologic examination of the stained tissue showed acute and focal endothelial denudation (J. Extra-Corporeal Technol. 21(2): 52-55, 1989).

Introduction

It has long been recognized that the velocity of flow during cardiopulmonary bypass (CPB) has associated potential deleterious effects. Galletti and Brecher discussed several undesirable aspects of high velocity arterial re-entry which included shear-induced blood trauma, cavitation, potential for vessel dissection, and suspected effects on vasomotion. Perfusionists are aware of the factors which are involved in high blood flow velocity and seek to minimize those hazards with proper cannula selection and maintenance of CPB conduct.

One area which perhaps has not been fully explored is the potential for high velocity aortic re-entry to induce endothelial cell damage adjacent to the cannulation site. It is clear from the literature that unusual flow patterns in the intact or disrupted circulation can indeed induce endothelial cell injury. It has been concluded that the vascular endothelium is particularly sensitive to local flow patterns. Low and high shear regions, vortex formation, and turbulent flow have all been implicated as causes of endothelial injury. Further, it is suggested that this form of injury could be as minimal as to simply induce an abnormal endothelial cell form or as serious as to denude the vessel of the endothelial cells and disrupt the smooth muscle layer.

The purpose of this study was to investigate the possibility that high velocity aortic re-entry and related events at the blood-endothelial interface will induce endothelial denudation adjacent to the cannulation site. Additionally, a qualitative assessment of endothelial cell damage during continuous flow versus pulsatile flow re-entry was made. The results of the study suggest that aortic endothelial cell denudation can result from arterial re-entry during CPB and that pulsatile re-entry may induce a more extensive damage.

Materials and Methods

Surgical preparation

Six mongrel dogs of 32.5 kg average weight (range 21.8-37.3 kg) were randomly divided into three study pairs. Pair one acted as control subjects (no CPB) while pairs two and three were studied with continuous and pulsatile flow CPB respectively. All subjects were initially anesthetized with 15 mg/kg Sodium Pentobarbital and intubated. Anesthesia was maintained throughout the study with a continuous 4 mg/kg/hr infusion of Sodium Pentobarbital via a right femoral vein catheterization. Positive pressure ventilation was established using a volume displacement ventilator initially set at a tidal volume of 15 ml/kg and a frequency of 12 breaths/min. The tidal volume and ventilatory frequency were adjusted throughout the pre-CPB phase of the study to maintain arterial pH within normal values. A standard three-lead electrocardiogram was monitored.

The right femoral artery was catheterized for arterial pressure measurement and blood gas sampling. A 7F thermodilution catheter was inserted in the right external jugular vein and flow directed into the pulmonary artery. This catheter was used to measure pulmonary artery pressure, central venous pressure, pulmonary capillary wedge pressure, blood temperature...
and cardiac output. Blood gases and pH were determined with a standard analyzer. Access to the chest was performed via a median sternotomy for experimental pairs two and three. Prior to cannulation for CPB, 300 u/kg Heparin was administered intravenously. Anticoagulation maintenance for the duration of the study was accomplished with intermittent Heparin administration to maintain the activated clotting time at 4x baseline value. Following verification of the initial coagulation, a 6.5 mm stainless steel tipped high flow aortic cannula was advanced into the ascending aorta for CPB arterial re-entry to the subject. A single 40 cm, 36-42 F cannulae was inserted into the right atrium for CPB venous return. A curved left heart vent catheter was advanced through the right superior pulmonary vein into the left ventricle immediately after CPB was done.

**Protocol**

One-half gram of Evans Blue Dye (EBD) diluted in 500 ml normal saline was infused over a one-hour period into all subjects. This infusion was started following the placement of the monitoring lines. Immediately following the cannulations of study pairs two and three, nonomthermic CPB was initiated at a cardiac index of 2.2-2.4 L/min. This event coincided with approximately 50 minutes of elapsed EBD infusion. The bypass circuit consisted of polyvinyl chloride tubing throughout. A roller pump delivered the blood from a bubble oxygenator, through an arterial line filter and into the subject via the aortic cannula. Each circuit was primed with two liters of heparinized (2.5 U/ml) lactated Ringers with 5% dextrose initially buffered to normal arterial pH values with Sodium Bicarbonate. Total CPB time for pairs two and three was kept constant at 80 minutes. Study pair three (pulsatile flow) started CPB with 20 minutes of continuous flow followed by 60 minutes of pulsatile flow. Pulsatile pumping parameters were kept constant at 50% pulse width, 20% baseline, and a pulse rate of 80. Collection of hemodynamic data was performed at ten minute intervals throughout the CPB period. Each data set included mean arterial pressure, central venous pressure, pump flow, and blood temperature.

Following the study period, each subject was sacrificed and the aorta was removed en bloc from the aortic valve to a point distal to the second arch vessel. The tissue was then sliced longitudinally and rinsed in saline. Color photographs of the segments were taken. Tissue samples were selected from EBD stained areas and adjacent dye free areas. Tissue samples were also taken in aortic segments completely free of EBD stain. All samples were preserved for later light microscope examination.

**Results**

All six subjects were maintained in acceptable hemodynamic status throughout the study period. Experimental pairs two and three tolerated the CPB procedure without incident. Mean arterial pressure for the continuous CPB pair (63.3 ± 18.4 mmHg) was significantly greater (p <.05) than the pulsatile CPB pair (50.8 ± 13.2). Central venous pressure, pump flow index, and temperature did not significantly differ between the two CPB pairs. Gross tissue examination of the aortic segments of the two control subjects (no CPB) revealed no visible EBD staining (Figure 1). Examination of the aortic segments of the CPB subjects revealed staining directly opposite from the cannulation site, around the origins of the arch vessels, and into the distal post-arch region. Qualitatively, it "appeared" that the pulsatile CPB pair stained more extensively and heavily than the continuous (non-pulsatile) CPB pair (Figures 2 and 3). Histological microscopic examination of the stained tissue indicated "focal loss of endothelium with a superficial area devoid of viable cells and loss and separation of elastic fibers very superficially in the aortic media". Importantly, the focal endothelial loss appeared "acute". Microscopic examination of the tissue clear of EBD stain (both control and CPB subjects) appeared "unremarkable with no intimal or medial changes".

**Discussion**

A key component of this study was the use of Evans Blue Dye as a marker of endothelial cell damage. This dye is a tetrasodium diazo organic salt which forms a strong bond to plasma proteins, particularly albumin. It has been shown that as endothelial permeability to plasma proteins increases (as in the case of cell damage), EBD is deposited from the albumin to tissue fixation points within the damaged vessel and thus serve to visibly mark the area of damage. It is possible that EBD in its free form (not associated with protein) also deposits in areas of endothelial cell damage. The depositing of EBD (and visibly observable staining) appears to be related to the extent of injury and the time allowed for exposure. The time of exposure in our application was sufficient to clearly mark areas of endothelial denudation.

Endothelial denudation, the native healing process, and its relationship to subsequent vascular disease has been extensively studied. A variety of methodologies have been used to denote regions of the vasculature including nylon catheter streaking, balloon catheter stripping, passage of bubbles, and coronary catheter manipulation. The end result of these manipulations is vascular endothelial denuding or endothelialization. Damage of a variety of widths and depths have allowed some comment toward the healing process and the potential for long-term compromise to endothelial cell layer continuity. Endothelial denuding may result in long standing...
endothelial abnormalities which could act as loci for advanced atherosclerotic vascular lesions. While the evidence is at times conflicting and difficult to explain, some factors involved in the post-denuding healing process have been identified. Subsequent atherosclerotic lesion development appears to be related to die2,21, the orientation of the denuded area with respect to axial flow, absolute blood flow through the vessel, and the length of time a vessel remains denuded15. Endothelial regrowth on denuded arteries appears to be limited and related to the size, shape, and depth of injury7,16,18.

Any extrapolation of the findings of this study to the clinical situation must be made with caution. The authors admit that the small sample size can only suggest preliminary observations. It is clear from the literature that the endothelium is particularly sensitive to high velocity stress and that de-endothelialization is implicated in certain circumstances with incomplete healing and atherosclerotic lesion formation. Our findings suggest that endothelial denuding can result from arterial re-entry during CPB. Qualitative differences between continuous and pulsatile flow are also suggested. Further studies must be undertaken to map the extent of endothelial denuding under the varied circumstances of arterial re-entry and CPB practice (e.g. cannula design, size, and orientation, vascular afterload, patient temperature, and bypass duration). Additional investigations should be performed to determine the implications on vascular lesion development.

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References

FIGURE 1: Photograph of one control (no bypass) aortic segment. Gross examination revealed no Evans Blue Dye staining.

FIGURE 2: Photograph of one continuous (non-pulsatile) bypass aortic segment. The segment is laid out with aortic valve annulus at the right, distal aorta at the left, and arch vessels at the top. Evans Blue Dye staining can be seen opposite the cannulation site (which is seen as a thin slice proximal to the arch vessels) and circling around the origin of the first arch vessel.

FIGURE 3: Photograph of one pulsatile bypass aortic segment. The segment is laid out with aortic valve annulus at the right, distal aorta at the left, and arch vessels at the top. Evans Blue Dye staining can be seen opposite the cannulation site (which is seen as a thin slice proximal to the arch vessels) and encircling the origins of both arch vessels.