Thrombosis in one coronary artery causes generalized coronary vasoconstriction in a dog model of unstable angina

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ABSTRACT

We investigated the effect of thrombosis in one coronary artery upon the vascular resistance of another coronary artery. In previous investigations, using an animal model of unstable angina, we have observed increased resistance downstream from thrombus within a left circumflex coronary artery (LCx) stenosis and vasoconstriction of collateral vessels from the left anterior descending artery (LAD) supplying the distal LCx vascular bed. In the present paper, we induced thrombosis within a stenosis of the LCx of 16 beagle dogs, and observed the changes in blood flow to the myocardium supplied by the LAD using the radioactive microsphere technique. This blood flow decreased with thrombus (P < 0.005) in these animals, whereas it did not do so in three time-control experiments. The pressures across the coronary vascular bed, i.e. arterial pressure to coronary venous pressure (coronary sinus catheter), did not change. Thus the vascular resistance of the LAD bed increased significantly from 147±11.5 mmHg/ml/sec/g of tissue to 172±13.4 mmHg/ml/sec/g of tissue (P = 0.02). As the LAD territory is not perfused with blood from the artery containing thrombus, we conclude that the effect observed is caused either by release of vasoconstrictors from the thrombus into the general circulation, or by activation of a neural reflex vasoconstriction. The study suggests that unstable angina involving thrombosis in one coronary artery is a global coronary vascular disease.

INTRODUCTION

We have studied the effects of intra-coronary thrombosis, using the dog model of Folts et al. [1], which simulates unstable angina, since thrombus forms and disperses, but does not completely occlude, the lumen. Initially, we observed changes in the pressure/flow ratio downstream from a thrombus in the left circumflex coronary artery (LCx) [2], and subsequently studied collateral flow through this bed distal to an LCx occlusion [3]. Both these studies indicated an increased resistance of the downstream LCx vascular bed. This could be attributed to the accumulation of emboli shed from the thrombosis site, although we did not observe a progressive increase in the phenomenon as predicted by this hypothesis. However, in a subsequent study without occlusion [4] but

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Abbreviations: LAD, left anterior descending artery; LCx, left circumflex coronary artery.
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with tight stenosis of the LCx, we observed that thrombosis caused an increase in resistance of the collateral vessels supplying the distal LCx vascular bed from the left anterior descending coronary artery (LAD) and right coronary artery. These vessels are not in the stream of blood flowing from the thrombus site, and cannot therefore be the conduit for emboli. We pos-
tulated that there was a generalized coronary vaso-
constriction in this situation. However, the collaterals are proximal vessels that do not directly feed the terminal coronary microcirculation. The present paper is the only one to present evidence of an increase in peripheral microcirculatory resistance in a coronary artery remote from that in which the thrombosis occurs.

When there is an occlusion or a stenosis of the LCx, blood goes through the LAD branch, where we can place a flowmeter, and right coronary artery. Some of the blood goes across the collaterals into the vascular bed normally supplied by the LCx, and some of it goes into the vascular bed normally supplied by the LAD. Therefore the flowmeter reading cannot be used to measure the vascular resistance of the anterior descending bed; however, this is readily achieved using a particulate tracer – in our case radioactive microspheres [5].

The purpose of the present study was to determine whether vasoconstriction could be discerned in the LAD vascular bed.

**METHODS**

Beagles (n = 16, weight range 11–18 kg) of either sex were premedicated with acepromazine (0.2 mg/kg) fol-
lowed by sodium pentobarbital (20 mg/kg). Supplemental barbiturate anaesthesia (3 mg/kg) was administered at 30 min intervals. Respiration was supported mech-

anically via a Manley volume cycled ventilator, with a 1:2
N\textsubscript{2}O/O\textsubscript{2} mixture adjusted with reference to blood gases and end-tidal CO\textsubscript{2}.

Following left thoracotomy, the LCx was dissected out as close to the bifurcation of the left coronary artery as possible for instrumentation with a perivascular ultrasonic flowmeter (Transonic Systems Inc., Ithaca, NY, U.S.A.). Additionally, a 22-gauge Teflon cannula (Abbocath; Abbott Ireland Ltd, Dublin, Ireland) was inserted into the LCx to allow measurement of distal circumflex coronary pressure. A tight artificial stenosis around the LCx was created between the flowmeter and the distal pressure cannula, using a 4 mm split polythene tube with a uniform internal diameter of 1 mm, secured in place with a silk ligature. Where necessary, additional narrowing or widening of the arterial lumen could be obtained by sliding of the thick end of a tapered nylon fishing line inserted between the wall of the artery and the polythene tube. The stenosis was deemed sufficient if the reactive hyperaemic response to transient circumflex arterial occlusion was abolished, and there was permanent reduction of basal coronary blood flow and distal coronary pressure when an LCx stenosis was imposed.

**Measurement of haemodynamic variables of pressure and flow**

Mean arterial pressure was measured via a cannula inserted into a femoral artery and connected via a fluid-filled line to a Statham P23Db pressure transducer. Venous access was via a femoral vein, with a 0.9% saline drip administered continuously to maintain blood volume. Coronary venous pressure was measured by insertion of a cannula into the coronary sinus via the jugular vein. Correct positioning was achieved radiographically, with verification by a blood gas measurement, and visually upon opening the chest. A standard three-limb ECG was measured continuously. Flow through the stenosis was measured directly from the flowmeter on the LCx. Analogue pressure and flow signals were amplified and fed into a MacLab/8s analogue-to-digital converter with built-in filters for signal conditioning. The digital output was then displayed and recorded on a Macintosh PowerBook computer running MacLab\textsuperscript{\textregistered} Chart version 3.5 software (AdInstruments Ltd, Castle Hill, New South Wales, Australia).

**Measurement of regional blood flow (radioactive microsphere technique)**

Regional blood flow measurements were achieved with the use of 15.5 ± 0.1 \( \mu \)m NEN-TRAC radioactive microspheres (NEN\textsuperscript{\textregistered} Life Science Products, Boston, MA, U.S.A.) suspended in 10% dextran and made up to volume (10 ml) with 0.9% saline solution. Two injections of approx. 3 \( \times 10^8 \) microspheres were labelled with \(^{141}\text{Ce},^{103}\text{Ru} \) or \(^{113}\text{Sn} \). The microspheres were admin-
istered into the left atrium. Before each injection the microspheres were sonicated and vigorously agitated to prevent clumping. Reference sample collection from a femoral artery at a rate of 13 ml/min was commenced 10 s before injection of the microsphere suspensions and continued for precisely 70 s.

On completion of the experimental protocol (described below), myocardium supplied by the coronary arteries was defined as follows. Barbiturate anaesthesia was deepened until the heart fibrillated. An aliquot of 15–20 ml of 15 \( \mu \)m yellow and blue fluorescent zinc/cadmium sulphide particles (Duke Scientific, Palo Alto, CA, U.S.A.), suspended in 0.9% saline, were injected into the LCx and LAD coronary arteries respectively. Care was taken to ensure that no back-flow occurred during the injection of the fluorescent spheres. The heart was removed, the surface wiped clean of blood and the

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cavities flushed with saline. The atria and right ventricles were removed and discarded. The left ventricle was fixed in 10% formaldehyde solution prior to analysis.

Tissue for radioactive counting was obtained by slicing the left ventricle into 4 mm transverse sections from apex to base. The regions of left ventricle supplied by the respective coronary arteries were separated after identification under UV light: LCx (yellow); LAD (blue); mixed tissue (green); other perfusion territories (colourless). The post-mortem injection of fluorescent spheres into the respective arterial beds allowed delineation of the perfusion area only of that portion of the vessel distal to the fluorescent sphere injection sites. The size of the beds is determined post-mortem using quantities of fluorescent microspheres which, if given during life, would cause pathological damage; such examination is not therefore repeatable in order to determine change in bed size with thrombosis. There is an indeterminate aspect to this because some areas do not fluoresce (e.g. areas supplied by the right coronary artery). Any areas of myocardium with no fluorescence were therefore discarded. Where such areas occurred, they accounted for less than 5% of total left ventricular mass. Others fluoresce green because they are supplied by both the LAD and LCx. In areas of myocardium where vessels from different arterial beds interdigitate, it is impossible to completely separate prospective regions of interest. In our tissue samples, these areas of mixed tissue, which appear green under UV light (yellow plus blue fluorescence), were discarded. However, the results apply to measurements away from these border areas, which are right in the centre of the LAD-supplied tissue where it would be unlikely for there to be such a great change in the border zone.

The different areas of each slice were cut into pieces, weighed and placed in plastic tubes for estimation of radioactive counts. Each tube contained at least 0.5 g of tissue, ensuring that no counted piece contained less than 400 spheres [6]. The accuracy of the measurement increases with the amount of tissue pooled [6], in this case all the blue tissue of the LAD area of supply. The radioactivity of the heart and reference blood samples was measured simultaneously in a Packard Autogamma Counter (model 5002/3). Myocardial blood flow values (ml/min per g) were calculated from the reference and myocardial sample counts after corrections for spillover from:

$$\text{Myocardial blood flow} = \text{myocardial counts} \times (\text{reference flow/reference sample counts})$$

**Experimental protocol**

**Stenosis**

The stenosis was tightened until basal circumflex flow had decreased from the pre-stenosis values by at least 10%, and was stable. At this time an appreciable stenotic pressure gradient (arterial pressure minus distal coronary pressure) could be observed. The control microsphere measurement was then obtained when all signals had stabilized.

**Stenosis plus thrombosis**

Endothelial damage of the region of LCx encircled by the polythene constrictor was achieved by crushing the artery with a haemostat, inducing platelet thrombus with the stenosis still in place. When three thrombus growth and embolization cycles were revealed on the LCx flowmeter [3], the second microsphere injection was made (never later than 30 min after the first).

**Time-control experiments**

Since it was always necessary for the thrombosis phase of the experiment to follow the control-stenosis-only phase, these periods could not be randomized. We reduced the time between the no thrombus and thrombus measurements to an absolute minimum, usually just 30 min. This was desirable for two reasons: (i) to minimize any possibility of a deteriorating preparation affecting our results; and (ii) to show that the observed effects during thrombosis are apparent a short time after damage of the artery is induced [i.e. after 3 cycles of thrombosis growth and embolization (see the Results section)]. To exclude the possibility that observed changes were time-dependent due to deterioration of the preparation over this short time, time-control experiments were performed in three beagles of similar weight range and age to the experimental group. Two microsphere measurements were made during 70 s occlusion of the LCx to ensure that collateral flow was in the LAD to LCx direction, as in the thrombosis experiments; the two measurements were 1 h apart.

**Expression of results**

The myocardial blood flow (Q) of the LAD-perfused territory (with blue fluorescence) was expressed in ml/min per g of tissue. The pressure gradient across the LAD vascular bed is the arterial pressure (P a) minus the coronary venous pressure (P v). The resistance of the LAD vascular bed was then expressed (in units of mmHg/ml/min/g of tissue) as:

$$\frac{(P_a - P_v)}{Q}$$

**Statistical analysis**

All differences between the flow and resistance values calculated during the stenosis and stenosis plus thrombus phases were analysed by Wilcoxon signed rank test.
Statistical significance was achieved at a probability value of $P < 0.05$.

**RESULTS**

**Time-control experiments**

No significant change was observed in mean arterial pressure or heart rate in all three time-control experiments (Table 1). Coronary haemodynamics were also constant, except during the coronary occlusion. No changes between the measurement corresponding to the two phases of the thrombosis experiments were discerned for LAD myocardial blood flow or resistance (Table 1) during a time which was double that for the experimental group.

**Effects of LCx stenosis on systemic and coronary haemodynamics**

The tight stenosis on a proximal segment of the LCx produced a pressure gradient between arterial pressure and distal LCx pressure in all experiments. This is also the pressure gradient across the collateral bed, and ensures that collateral flow is from the LAD to the LCx, and, therefore, the LAD vascular bed cannot be in the stream of blood coming through the LCx stenosis. When a steady-state had been achieved, the first microsphere label was injected. The stenosis did not cause any discernible general systemic effects as evidenced from records of mean arterial pressure and heart rate. The values for all measured variables during this period are shown in Table 1.

**Effects of platelet thrombosis on coronary arterial flow and resistance**

**Proximal circumflex flow**

Endothelial damage at the site of constriction of the circumflex artery resulted in episodic platelet aggregation and embolization, which was manifested as cyclic reductions in LCx blood flow [7]. The LCx thrombosis did not cause any discernible general systemic effects as evidenced from records of mean arterial pressure and heart rate. Growth of a platelet-rich thrombus at the stenosis was accompanied by a progressive increase in resistance across the stenosis, limiting flow. This persisted until embolization of the thrombus (removal of the lumen obstruction), when the stenosis resistance decreased rapidly as flow was restored. There were no changes in systemic haemodynamics during cyclic flow reductions (Table 1).

**Anterior descending flow and resistance**

Regional blood flow to myocardium perfused by the LAD was significantly reduced after establishment of the thrombus within the LCx stenosis (Figure 1). Arterial and coronary venous pressure were unaffected (Table 1).

| Table 1 | Paired measurements of key variables in stenosis and stenosis plus thrombosis ($n = 16$) and time-control experiments ($n = 3$) |
|------------------|-----------------------------------------------|-----------------------------------------------|
| Heart rate       | Coronary blood flow                          | Coronary vascular resistance                  |
|                  | (beats/min)                                   | (ml/min per g of tissue)                      |
|                  | $P_A$ (mmHg)                                 | $P_V$ (mmHg)                                 |
|                  | $P_A - P_V$ (mmHg)                           | $P_A - P_V$ (mmHg)                           |
|                  | $P_A - P_V$ (mmHg)                           | $P_A - P_V$ (mmHg)                           |
| Stenosis         | $162 \pm 5.7$                                 | $111 \pm 3.0$                                |
| Stenosis + thrombus | $163 \pm 4.9$                                | $107 \pm 3.0$                                |
| Time-control 1   | $154 \pm 13.7$                                | $109 \pm 0.6$                                |
| Time-control 2   | $163 \pm 23.6$                                | $103 \pm 3.5$                                |

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Figure 2 Effect of coronary thrombosis in the circumflex artery upon vascular resistance of the anterior descending artery bed.

The resistance of the vascular bed of myocardium in the LAD region of supply is shown (n = 16). This was calculated from mean arterial pressure minus coronary venous pressure divided by regional LAD flow (ml/min per g). Left, control period (no thrombus); right, during thrombosis in the parallel LCx (thrombus). The lines connect the pairs of resistance measurements of each individual experiment. P is the probability of no difference in resistance for all experiments by Wilcoxon signed rank test.

Therefore the LAD resistance was significantly increased (Figure 2).

DISCUSSION

The present paper shows that thrombosis within the stenosis of one coronary artery causes vasoconstriction of the vascular bed of another coronary artery. The other coronary artery, in this case the LAD, is not in the stream of blood coming from the thrombus and collateral flow is directed away from the LAD.

In order to eliminate the possibility of deterioration of the experimental preparation introducing error between the first and second set of measurements, we performed three time-control experiments in the absence of stenosis or thrombosis. These studies demonstrated no changes in LAD flow or resistance. A power calculation showed that any increase in the value of n, from n = 3 to any practical value, would not lead to a statistically significant difference between simulated thrombus and non-thrombus phases. For ethical reasons we therefore refrained from further experiments.

As the arterial pressure was unaffected by the presence of coronary thrombosis, we incline to the opinion that the coronary vasoconstriction is not part of a general systemic vasoconstriction. Clearly, the wide-ranging experiments required to elucidate the mechanism are beyond the scope of the present study. The problem with investigations of potential vasoconstrictors, such as catecholamines and serotonin, is that their antagonists also disperse the thrombus, so that reversal of vasoconstriction would be difficult to interpret. Possibly, tiny amounts of antagonists could be infused intrarterially in doses that would be negligible upon recirculation. The neural hypothesis would require experiments either (i) on regionally denervated hearts (e.g. with the area supplied by the LCx and that supplied by the LAD innervated), or (ii) globally denervated hearts, in which the phenomenon is predicted to disappear according to the postulate.

It would be of interest if our observations of collateral vessel constriction with thrombosis [4] are applicable to humans, since restricted collateral flow would have a deleterious effect if it occurred in unstable angina. The collaterals are proximal vessels that do not directly feed the terminal coronary microcirculation. The present paper is the only one to present evidence of an increase in peripheral microcirculatory resistance in a coronary artery remote from that in which the thrombosis occurs. All the evidence thus far on this ‘Folts’ model indicates that it gives a very accurate simulation of unstable angina patients [1,2,7–10]. The ischaemia induced by intraluminal thrombosis during unstable angina would be intensified by the presence of generalized coronary vasoconstriction.

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