Native Collaterals in the Development of Collateral Circulation After Chronic Coronary Stenosis in Mongrel Dogs

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SUMMARY The response of native collateral circulation to chronic stenosis of the left circumflex coronary artery (LCx) was studied in 17 mongrel dogs. Stenosis restricted reactive hyperemia of the LCx without affecting resting flow. Regional myocardial blood flow was measured by the tracer microsphere technique. Coronary collateral blood flow to the LCx was determined during maximal reactive hyperemia of the left anterior descending branch before and 5 weeks after implantation of a fixed LCx stenosis in the open-chest preparation. The protective effect of collaterals was tested by LCx ligation 5 weeks after implantation of stenosis. Presence of acute myocardial infarction was determined by nitroblue tetrazolium staining. Eleven dogs had a myocardial infarction (group A), but six dogs showed no evidence of infarction at autopsy (group B). In group A, collateral flow and minimal coronary resistance of the LCx bed changed little after LCx stenosis, from 12 to 15 ml/min/100 g and from 10.5 to 10.0 mm Hg/ml/min/100 g, respectively (both \( p > 0.05 \)). In contrast, collateral flow in group B increased from 22 to 102 ml/min/100 g (\( p < 0.05 \)), and minimal coronary resistance of the LCx bed decreased from 4.8 to 1.4 mm Hg/ml/min/100 g (\( p < 0.01 \)). Group A had lower native collateral flow (\( p < 0.05 \)) and higher native minimal coronary resistance of the LCx bed than group B (\( p < 0.05 \)). Postobstructive LCx pressure correlated well with blood flow data. The LCx risk region was of comparable size in groups A and B, 36.4% vs 39.0% of total left ventricle (\( p > 0.05 \)).

Two responses of collateral circulation to chronic stenosis were documented: lack of collateral growth in group A, but significant collateral growth in group B. The natural variation of collateral circulation was the major determinant of the different responses that were important with stenosis of a major coronary artery.

THE AMOUNT of collateral blood flow available in ischemic areas of the myocardium after experimental coronary artery occlusion has a protective effect on the myocardium and limits or even prevents infarction.1, 2 After chronic coronary artery occlusion, collaterals enlarge considerably.1-4 The degree of compensation by collaterals for the loss of the occluded native circumflex branch of the left coronary artery (LCx) in mongrel dogs was 33% of its former conductance.5 Similar quantitative data regarding collateral development after chronic coronary stenosis are lacking. Elliot et al.5 showed in dogs that the mean peripheral LCx pressure increased before the coronary artery was totally occluded, but the major rise of peripheral LCx pressure occurred only when resting flow decreased markedly. In a more recent study by the same group, coronary blood flow of small branches of the LCx was monitored.4 Collateral flow began to rise during the phase of severe coronary stenosis. However, no systematic information is available about the response of collateral circulation to a coronary stenosis that does not affect resting flow but restricts reactive hyperemia. In addition, it is not known whether the natural variation of native collaterals influences the development of collaterals in response to coronary stenosis. The purpose of the study was to define whether collaterals develop after chronic stenosis of the LCx, which restricts reactive hyperemia without affecting resting blood flow, and to investigate whether the natural variation of collaterals modifies collateral growth.

Methods

The experiments were carried out in 17 mongrel dogs of either sex, of unknown age with an average body weight of 21 kg.

Surgical and Experimental Procedures

A left thoracotomy was performed using aseptic techniques and anesthesia with subcutaneous piritramide, 5 mg/kg body weight and i.v. sodium pentobarbital, 10 mg/kg. Anesthesia was maintained with 80% nitrous oxide and 20% oxygen under intermittent positive pressure respiration with a Bird Mark 7-Mark 4 combination. Aortic pressure was measured with a #8F catheter (femoral artery) connected to a Statham pressure transducer. ECG lead II and aortic pressure were recorded on a Siemens ink-jet recorder. The LCx just distal to its first small marginal branch and the left anterior descending branch (LAD) proximal to the septal branch were exposed by blunt dissection, and loose ligatures were placed around them. A #6F catheter was introduced into the left atrium. Regional myocardial blood flow was measured (tracer microsphere technique) during acute occlusion of the LCx and 10 seconds after release of a 60-second occlusion of the LAD (maximal reactive hyperemia). This flow determination represents maximal native collateral flow to the LCx. Thereafter, LCx flow was monitored using an electromagnetic flowmeter (Statham). The LCx was narrowed with a Teflon ring (i.d. 0.8–1.5 mm) that reduced the maximal reactive hyperemia of the LCx to

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about 50% of control value (no stenosis) but did not change resting coronary blood flow. Finally, pressures in the LCx and the aorta were measured simultaneously during a 60-second LCx occlusion (10 dogs). The chest was closed and dogs were allowed to recover.

Five weeks later, the dogs were anesthetized and underwent another thoracotomy. The LCx was ligated and regional myocardial blood flow was determined 10 seconds after release of a 60-second occlusion of the LAD (collateral flow after chronic LCx stenosis). Pressures in the LCx and the aorta were measured simultaneously (10 dogs). After 4 hours of LCx ligation, followed by 1 hour of reperfusion, the dogs were killed and their hearts removed. The right ventricle was dissected from the left ventricle. The left ventricle was cut into five transverse rings 1.2 cm thick from base to apex. The rings were incubated in nitroblue tetrazolium for 30 minutes (0.25 g/l, 0.1 M Sorensen’s phosphate buffer at pH 7.4, and 0.1 M malate). Normal myocardium stains blue in the presence of dehydrogenases, while infarcted myocardium, which is depleted of dehydrogenases, does not stain. The borders of the infarcts were clearly delineated in each ring.

Tracer Microsphere Technique

Regional myocardial blood flow was measured with radioactive tracer microspheres, 10 ± 2 µ in diameter (3M Company) with Tween-80 and labeled with five isotopes (125I, 141Ce, 85Sr, 95Nb and 46Sc). The microspheres were stirred mechanically for 30 minutes and ultrasonicated (50 W) for 1 minute immediately before each injection. Five million microspheres were injected over 10 seconds through the left atrial catheter. A reference arterial blood sample was withdrawn for exactly 1 minute beginning 5 seconds before each injection at a constant rate of 20 ml/min for calculation of flow (ml/min per 100 g of myocardium) by the reference sample method. Each slice of the left ventricle was unrolled and divided into sections counterclockwise from the anterior free wall (fig. 1). Each transmural tissue section was further divided into a subendocardial (endo), intermediate and subepicardial (epi) sample (fig. 1). Each sample was accurately weighed (average 500 mg), coded, and transferred to plastic tubes. The tubes were automatically transported by a Selektrom sample changer into and out of a 3-inch, NaI, well-type scintillation crystal. The transport was controlled by a ND 812 8K 12-bit process computer. The compound gamma spectrum of the radionuclides present in the tissues was analyzed by the ND 812 process computer. Background correction and spillover produced by the Compton scattering were taken into account. Details of the computer program have been published. Corrected activities were expressed as counts/min/mg. The data were printed out on a teletype printer and punched in parallel on paper tape, which was fed into a PDP 11-45 computer for further data processing and graphic presentation. To identify the LAD and LCx perfusion areas, the borders were demarcated in each ring on the blood flow mappings. Samples between both perfu-
mappings during acute LCx occlusion. (The weights of samples with low flow were summed for each ring.) The infarct size was expressed as infarct weight divided by weight of LCx perfusion area.

Statistics

For statistical analysis the t test was used. Values are mean ± SEM in the tables and figures.

Results

Group A included 11 dogs who had a fresh myocardial infarction at autopsy. Group B included six dogs without myocardial infarction. Four other dogs died about 2 weeks after LCx stenosis and another four died of irreversible fibrillation after ligation. Data from these eight dogs were discarded.

Regional Myocardial Blood Flow

In group A, myocardial blood flow, the endo/epi ratio and coronary vascular resistance remained unchanged after LCx stenosis in the LAD as well as the LCx perfusion area. An example of blood flow mapping of a group A dog is presented in figure 3, which shows a lack of augmentation of LCx flow. In group B, blood flow increased in the LCx (table 1). An example of blood flow mapping of a group B dog is shown in figure 4, which shows that LCx collateral flow increased after LCx stenosis. Figure 5 is a summary of changes of collateral flow and minimal coronary resistance of LCx bed for both groups. Significant changes occurred after LCx stenosis in group B. Native collateral flow was higher and minimal coronary resistance was lower in group B than in group A. These differences became more pronounced after LCx stenosis (table 1).

The left ventricular weight was 89.9 ± 8.7 g (range 48.7–139.9 g) in group A and 93.6 ± 4.2 g (range 78.6–106.9 g) in group B (p > 0.05). The weight of the LCx risk region was 32.8 ± 3.2 g (range 17.9–48.0 g) in group A and 36.8 ± 3.1 g (range 22.8–42.8 g) in group B (p > 0.05). The risk region as a percentage of the left ventricle was 36.4 ± 1.0% (range 30–42%) in group A and 39.0 ± 2.4% (range 29–44%) in group B (p > 0.05). The infarct mass as a percentage of the LCx risk region was 44.1 ± 6.4% (range 18–77%) in group A.

Hemodynamics

The reactive hyperemic response after implantation of LCx stenosis was similarly reduced, to 59.2 ± 2.6% of control in group A and to 54.8 ± 2.2% in group B (p > 0.05). At the second study, all dogs in group A and two of six dogs in group B revealed residual lumen of LCx stenosis at autopsy. Four dogs of group B showed complete occlusion of LCx stenosis. LCx pressure (measured in six dogs of group A and four dogs of group B) remained unchanged in group A but increased in group B after stenosis (p < 0.05) (table 2). LCx pressure was higher in group B than in group A, but the difference was significant (p < 0.05) only after stenosis. Heart rate and mean aortic pressure were the same before and after stenosis.

Discussion

The study shows two responses of collateral development to chronic coronary stenosis: a lack of collateral growth in group A and significant development of collateral circulation in group B. The major determinant for the two responses was the level of native collateral flow as measured at the initial study. Since the LCx perfusion areas were comparable in both groups, we could not attribute the difference of native collateral circulation to the size of the LCx bed. The development of collaterals was measured by an increase of flow to the LCx bed, a fall of coronary resistance and a rise of postobstructive LCx pressure in group B. In contrast, no change of these variables was observed in group A after an identical period of coronary stenosis. The experiments discovered two groups of dogs that demarcate the natural range of variation in native collateral circulation of mongrel dogs. A re-
Markable variation of native collateral circulation was shown between collateral flows of mongrel dogs and pigs: Dogs had a threefold higher native collateral flow than pigs. Whether an inborn variability of collateral circulation affects the natural history of patients with coronary artery disease is unknown. In chronic coronary artery occlusion of patients with coronary artery disease, a great variability of collaterals was documented by several studies. The development of these vessels during coronary stenosis and occlusion of the native coronary artery was not systematically studied in patients.

The fate of the postobstructive myocardium after coronary occlusion is determined by the adequacy of coronary collaterals. A well-developed collateral circulation after chronic coronary occlusion protects the myocardium against tissue damage in the majority of mongrel dogs. Normal resting blood flow was measured in the collateral-dependent area, but flow remained restricted during stress or vasodilation. A decrease of coronary collateral resistance after chronic coronary occlusion was first measured by Flameng et al. Respective data after chronic coronary stenosis have not been published. In the present study, a fixed coronary stenosis was used instead of a chronic coronary occlusion. Chronic coronary occlusion was produced with an ameroid constrictor, which initially is nonobstructive but gradually occludes (2-3 weeks after implantation) the coronary vessel after absorbing fluid. The Teflon ring used in the present study did not change after implantation (as shown at autopsy) and did not occlude the coronary artery completely. The degree of stenosis was defined during reactive hyperemia and reduced the maximal reactive hyperemia to 55-60% of the control value. This degree of stenosis equals an 80% diameter reduction as estimated from the studies of Gould et al. Despite this degree of LCx stenosis, no growth of collaterals could be detected in

![Figure 4. Blood flow mapping of a ring of a dog heart from group B. After chronic left circumflex coronary artery (LCx) stenosis, collateral flow increased (B) compared with the flow before chronic LCx stenosis (A). LAD = left anterior descending coronary artery; TZ = transitional zone; endo, inter and epi = endocardial, intermediate and epicardial zones.](image-url)
group A. This lack of collateral growth despite chronic stenosis is a new finding. Flow through the stenosis was normal at rest in all dogs at first study. Since stenosis did not progress, myocardial ischemia may have been present only during stress or physical exertion. This degree of ischemia (transient ischemic episodes) did not sufficiently promote growth of collaterals in group A. In contrast, the same conditions induced significant collateral development in group B. Caging and daily activity were the same for both groups. The degree of restriction of reactive hyperemia after stenosis, the duration of chronic stenosis and the size of the perfusion area were also the same.

The only difference between group A and B was the level of native collateral flow (and native minimal coronary resistance of LCx bed). The same amount of coronary constriction produced two responses of collateral growth. No dog of group A, but four of six dogs of group B, revealed spontaneous coronary occlusion at second study. Since group B started with a higher collateral flow than group A, one can assume that collateral flow itself induced progression of stenosis. Previous studies showed that flow may subside across a coronary stenosis when collateral flow is high. This functional occlusion (zero flow despite residual vessel lumen) may lead to final thrombotic occlusion. In several dogs of group B, the coronary stenosis was investigated by light microscopy and an occluding thrombus was detected in the residual vessel lumen (Schaper J: unpublished observations).

Presence of myocardial infarction was assessed using nitroblue tetrazolium. This technique provides an accurate delineation of infarcted tissue in ventricular myocardium. The validity of the nitroblue tetrazolium technique for detecting early myocardial infarction in fresh myocardial slices has been shown. The accuracy of the tracer microsphere technique for measuring regional myocardial blood flow to small tissue samples under conditions of reduced flow has been confirmed. Buckberg et al. observed that if the number of spheres fell below 400 in simultaneously collected reference blood samples, the random variability in the number of spheres increased precipitously. Similar variations in measuring organ blood flow would occur when tissue samples contained less than 400 spheres. We injected 5 million microspheres. If cardiac output were estimated at 2000 ml/min and blood volume at 2000 ml, withdrawal of the reference blood sample at a rate of 20 ml/min (1% of cardiac

TABLE 2. Hemodynamic Data

<table>
<thead>
<tr>
<th></th>
<th>Group A (n = 11)</th>
<th>Group B (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Native</td>
<td>After stenosis</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>116 ± 8</td>
<td>114 ± 7</td>
</tr>
<tr>
<td>Mean aortic pressure (mm Hg)</td>
<td>94 ± 7</td>
<td>95 ± 5</td>
</tr>
<tr>
<td>LCP d/AOP d</td>
<td>0.09 ± 0.01*</td>
<td>0.11 ± 0.01*</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

*Six observations.
†Four observations.
‡p < 0.05 compared with group A.

Abbreviation: LCP d/AOP d = ratio of diastolic left circumflex pressure and diastolic aortic pressure.
output) for 1 minute, the sample would collect 50,000 spheres. Furthermore, if we measure a collateral blood flow of 10 ml/min/100 g in the LCx bed of about 40 g (in left ventricle of 100 g) the LCx bed would contain 50,000 spheres and a tissue sample of 0.5 g, 625 spheres. Thus, for measuring low native collateral flow with the technique used in the present study (weight of myocardial sample = 0.5 g), injection of 5 million microspheres gives accurate results. This is also shown empirically by the original blood flow mappings in figures 3 and 4, which reveal only minimal scatter of blood flows in the low-flow LCx area.

Capurro et al. and Jugdutt and Becker reported a significant loss of microspheres from infarcted myocardium 1, 2, 4 and 8 days after acute permanent coronary occlusion when the spheres were injected before coronary occlusion. However, Capurro et al. reported no loss after short, transient coronary occlusion or when coronary collateral blood flow was high after permanent coronary occlusion. Further, the microspheres remained undisturbed in the myocardium provided the myocardium remained viable. In the present study, the coronary artery was occluded for only 1–2 minutes, and myocardial necrosis did not occur between first and second study. The data presented in table 1 reveal identical myocardial blood flows and, hence, microsphere content, in intact myocardium perfused by high or low flow rates during microsphere injection. Thus, there is no evidence that loss of microspheres occurs from viable myocardium up to 5 weeks.

In conclusion, the level of native collateral flow was the major determinant of collateral growth. This may also be the case in patients with acute myocardial infarction. The significance of coronary collateral circulation in this clinical situation has been shown.

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Native collaterals in the development of collateral circulation after chronic coronary stenosis in mongrel dogs.
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