Increase in Collateral Blood Flow Following Repeated Coronary Artery Occlusion and Nitroglycerin Administration

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SUMMARY. The effects of occlusion, reperfusion, reocclusion (n = 13), and nitroglycerin (n = 10) on regional transmural myocardial collateral blood flow was tested in conscious dogs in which collateral development was stimulated by partial stenosis of the left circumflex coronary artery. Hemodynamics and collateral blood flow were measured during the awake state using 9-μm radioactive microspheres. Regional transmural flow was measured during transient occlusion of the circumflex artery at 7 and at 14 days postoperatively. On the 14th postoperative day, two sets of circumflex occlusions and blood flow measurements were carried out. The first set consisted of two occlusions separated by 15 minutes. The second set performed 2 hours later included two occlusions, separated by 15 minutes, and nitroglycerin administration. Mean collateral blood flow increased significantly (P = 0.002) from 0.10 ± 0.07 ml/min per g on day 7 to 0.25 ± 0.18 ml/min per g on day 14. A significant increase in mean collateral blood flow from occlusion one to two was observed (0.28 ± 0.17 to 0.37 ± 0.22 ml/min per g, P = 0.005). Mean collateral flow increased significantly (P = 0.01) between pre- to post-nitroglycerin occlusions, 0.28 ± 0.20 to 0.46 ± 0.32 ml/min per g. Although this increase appeared to be greater than during the first set of occlusions, it did not reach statistical significance (P = 0.08). These data indicate that when immature collaterals are present, occlusions, reperfusion, and reocclusions of a major coronary artery produce augmentation in collateral flow. This must be considered in evaluating interventions which may alter collateral flow. (Circ Res 54: 204-207, 1984)

IN the event of coronary artery occlusion, collateral vessels can provide an alternate source of blood flow. Little is known, however, about the functional physiology of immature collateral vessels. If a partially stenosed major coronary artery is suddenly totally obstructed, the integrity of the myocardium will become entirely dependent upon the functional adequacy of the collateral vessels. It is important, therefore, to determine whether immature collateral vessels function to deliver a fixed maximal amount of flow immediately after total occlusion of the stenosed coronary, or if blood flow to the collateral-dependent area can be augmented by prior brief episodes of total occlusion, or by drug administration.

In order to carry out these studies, a model was developed in our laboratory in which collateral vessel growth was stimulated by partial stenosis of the left circumflex coronary artery. In our model, the blood flow to a region of myocardium was supplied by both the partially stenosed circumflex artery, and by immature but developing collateral vessels. During complete temporary occlusion of the circumflex artery, the immature collaterals were the primary source of flow to this area of myocardium.

The primary aim of the study was to determine whether (after a brief occlusion of a major coronary artery and reperfusion), a second occlusion would elicit equal, increased, or decreased collateral flow in addition, to provide a basis for evaluating the results of studies in which multiple coronary occlusions are performed to compare the effects of drugs on collateral perfusion. A second aim was to determine whether nitroglycerin could augment flow to an area of myocardium supplied by immature collateral vessels. These data will have important implications in a complete understanding of the physiology of the collateral circulation.

Methods

Fourteen adult mongrel dogs weighing 16–27 kg were anesthetized with intravenous sodium thiopental (30 to 40 mg/kg), and underwent a left thoracotomy, using sterile technique. Since the integrity of the study was dependent upon complete occlusion of the circumflex, the proximal left circumflex coronary artery was dissected free and an electromagnetic flow probe (Howell Instruments Inc.) positioned around the vessel in order to verify zero flow during occlusion. A pneumatic occluder constructed in our laboratory was placed distal to the flow probe. The occluder diameter was adjusted as snug as possible without altering the reactive hyperemic response to a 10-second coronary occlusion. Polyvinyl chloride heparin-filled catheters, 3 mm in diameter, were placed in the ascending aorta via the left internal mammary artery, the left atrial...
appendage, and the left ventricle, through a stab wound in the apex. The catheters, occluder, and flow probe leads were tunneled through the chest wall, placed in a subcuta-
naneous pouch at the base of the neck, and the thoraco-
tomy was closed. Routine postoperative care, including
antibiotics, was followed. The animals were placed in the
vivarium to recover.

The animals were brought to the laboratory 7 days
postoperatively. Morphine sulfate (10–20 mg) was admin-
istered intramuscularly. By sterile technique, the subcuta-
naneous pouch was infiltrated with 2% lidocaine hydrochlo-
ride, an incision made, and the instrumentation exterior-
ized. The aortic, left ventricular, and left atrial pressure
catheters were connected to Statham P23Db transducers.
The flow probe was connected to a Howell model HMS
1000 flowmeter (Howell Instruments). After 1 hour or
more, phasic aortic, left ventricular, and left atrial pres-
sures were monitored continuously and recorded, as were
phasic left circumflex coronary artery flow and lead II of
the electrocardiogram, on a Hewlett-Packard model 7700
eight-channel direct-wiring oscillograph. The pneumatic
occluder was inflated to produce complete occlusion of
the left circumflex artery, and continuously verified by the
flowmeter. Two minutes after the onset of occlusion, a
regional transmural myocardial blood flow measurement
was carried out with radioactive microspheres. The occlu-
sion was maintained until the reference sample collection
was completed, then was released. The instruments were
disconnected and replaced into the subcutaneous pouch.

The animals again were brought to the laboratory on
the 14th postoperative day for study. The subcutaneous
pouch was reopened and the instrumentation was con-
nectec as described previously. After a control basal state
was attained, hemodynamic data and regional transmural
myocardial blood flow measurements were obtained.
Then the coronary artery was occluded for approximately
4 minutes (the microspheres injected at 2 minutes). Fifteen
minutes later, the occlusion was repeated, adhering to the
identical protocol. A period of 2 hours elapsed. Again, the
coronary artery was occluded for approximately 4 minutes in
duration, and the same parameters were measured. Ten
minutes later, 0.4 mg nitroglycerin (Parke-Davis Corp.),
freshly dissolved in 5 ml of normal saline, was adminis-
tered intravenously over a 30-second period and flushed
over 30 seconds with 10 ml of normal saline. Approxima-
ately 5 minutes following this nitroglycerin administra-
tion, after hemodynamics had returned to baseline, again
the coronary artery was occluded for approximately 4
minutes, following the protocol exactly.

Regional transmural myocardial blood flow was meas-
ured with 9 ± 1-μm microspheres (Minnesota Mining and
Manufacturing Co.) labeled with six different γ-emitting
radionuclides, as has been described previously (Swain et
al., 1979).

After completion of all data collection, the animals were
anesthetized with sodium thiamylal and killed with po-
tassium chloride. Each of the 14 hearts was placed in 10%
buffered formalin, fixed, trimmed, and sectioned into six
anatomic regions, as described previously (Fedor et al.,
1980). Each region was subdivided into four transmural
layers from epicardium (layer 1) to endocardium (layer 4).

Gross inspection and histological examination of appro-
priate samples revealed no evidence of myocardial inar-
tion. The individual tissue samples weighing from 0.5 to
1.5 g were placed into separate formalin-filled vials for
subsequent counting.

The anatomic region receiving the least blood flow
during circumflex coronary artery occlusion on postoper-
ative day 7 was designated the ischemic or collateral-
dependent region, assuming this area most dependent
upon the developing collaterals for blood flow. This was
most often the posterior papillary region. The normally
perfused or nonischemic regional flow was obtained as
averages from the anterior and anterior papillary regions.

Hemodynamic data were measured directly from the
oscillograph recording obtained at the time of microsphere
injection. Data in the Results section are expressed as
mean ± 1 so. Statistics were based on Student's t-test for
paired data, analysis of variance, and linear regression.

Results

The 13 dogs studied on postoperative day 7, and,
again, on postoperative day 14, showed an increase
in blood flow to the collateral-dependent region
during circumflex occlusion between the two meas-
urements. These dogs were considered to have col-
ateral development stimulated by the partial ste-
nosis of the left circumflex coronary artery. All myo-
cardial blood flow changes are greater than the error
associated with the microsphere method for this
laboratory (Swain et al., 1979). Mean flow to the
collateral-dependent region increased from 0.10 ±
0.07 ml/min per g on day 7 to 0.25 ± 0.18 ml/min per
g on day 14 (P = 0.01). Mean flow to the
normally perfused myocardium was 1.04 ± 0.42
ml/min per g on day 7, 1.4 ± 0.64 ml/min per g on
day 14, and was not different (P = 0.59). Hemody-
namic parameters during occlusion were not statisti-
cally different on postoperative days 7 and 14.

The effect of occlusion, reperfusion, and reocclu-
sion on postoperative day 14 in 13 dogs is shown in
Figure 1. The repeat occlusion data for one dog was
not useable because of technical difficulties with the
blood flow measurements. Mean blood flow to the
myocardium supplied by immature collateral vessels
increased significantly from 0.28 ± 0.17 to 0.37 ±
0.22 ml/min per g from occlusion 1 to occlusion 2
(P = 0.01). Flow increased significantly to each layer
(P = 0.04). However, the magnitude of this increase
to each of the four layers was not statistically differ-
ent (P = 0.58). The endo/epi increased significantly:
0.23 ± 0.15 to 0.32 ± 0.17 (P = 0.01). Hemodynamic
data were not different during each of the occlusions
(see Table 1). Mean flow to the noncollateral-de-
pendent region was 1.12 ± 0.65 ml/min per g during
occlusion 1 and 1.12 ± 0.21 ml/min per g during
occlusion 2.

After 2 hours, repeat occlusion in 10 dogs dem-
onstrated that mean blood flow and the endo/epi
were no different from that of occlusion, 1 of the
first set of occlusions indicating that vascular reac-
tivity had returned to baseline (Fig. 1). Hemody-
namic measurements during these occlusions were
similar (see Table 1).

The effect of administration of nitroglycerin in 10
dogs is shown also in Figure 1. Using pre-nitro-
glycerin flow as the control, mean flow in the collateral-dependent region was found to increase significantly from 0.28 ± 0.20 to 0.46 ± 0.32 ml/min per g (P = 0.01). Flow to layers 2, 3, and 4 increased significantly (P = 0.01). Flow to layer 1 was 0.49 ± 0.25 ml/min per g during pre-nitroglycerin occlusion and 0.53 ± 0.27 ml/min per g during post-nitroglycerin occlusion, the increase not being statistically significant (P = 0.63). The endo/epi increased significantly from 0.25 ± 0.14 to 0.58 ± 0.27 (P = 0.01). In order to determine whether nitroglycerin produces an independent effect, flow measured during occlusion 2 of the first occlusion set was used as the control. The change in mean flow tended to be greater but did not reach statistical significance (P = 0.08). However, increase in flow to transmural layers 2, 3, and 4 following nitroglycerin was significantly greater than with repeated occlusion alone (P = 0.04). The endo/epi increased significantly more (P = 0.01) between pre- and post-nitroglycerin occlusions, compared with the first set of occlusions. In the normally perfused region, mean flow decreased significantly from 1.06 ± 0.33 ml/min per g during pre-nitroglycerin to 0.82 ± 0.34 ml/min per g during the post-nitroglycerin occlusion (P = 0.01), while the endo/epi in this region remained the same. Hemodynamic measurements are listed in Table 1.

Two dogs were studied in the reverse order (performing the nitroglycerin set of occlusions first), and no difference was found between responses of these dogs and the others.

**Discussion**

The canine model developed in this investigation utilized a partial coronary stenosis to stimulate the development of collateral perfusion rapidly. The technique employed is similar to that described by Cohen and Yipintsoi (1979). Since flow can be acutely interrupted in the coronary vessel at the point of stenosis, the function of the collateral vessels during transiently augmented flow can be evaluated, i.e., flow measured in the collateral-dependent area is due to flow through collateral vessels. The posterior papillary region was most often selected, since it had the lowest flow of the regions perfused by the left circumflex coronary artery, and has been shown to have a high correlation between area at risk from circumflex occlusion and infarction area (Koyanagi et al., 1982). Flow to all layers in the ischemic region did increase significantly from day 7 to day 14, and from occlusion one to occlusion two, while nonischemic flows remained the same. The question arises whether the increase in blood flow to the collateral-dependent area after 7 days might be related to the preferential loss of microspheres. This has been described in infarcted but not non-infarcted areas of myocardium (Murdock and Cobb, 1980). Since the collateral-dependent region in the present study was not infarcted, the increase in flow to the collateral-dependent region is not likely to be a result of microsphere loss.

Total occlusion, reperfusion, and reocclusion after 15 minutes demonstrated clearly increased flow to all layers of collateral-dependent myocardium. Thus, some effect of the first occlusion stimulated further collateral flow during the subsequent occlusion. Hemodynamic parameters were similar during
both occlusions and, therefore, could not have been responsible for the flow increase.

That collaterals do not open immediately when stimulated was suggested by earlier studies of Greenfield et al. (1972). Following acute interruption of flow in a vein graft, reactive hyperemia was markedly attenuated as the time of occlusion increased. These observations suggested that existing collaterals on which the myocardium was dependent had begun to close after the insertion of the vein graft, and did not open immediately when this new source of blood supply was acutely interrupted. Information concerning the time course of this phenomenon can be obtained from the pre-nitroglycerin occlusion performed 2 hours after release of the second occlusion and reperfusion. Hemodynamics were similar during the second and third occlusions, and mean flow and endo/epi were not statistically different. Thus, in 2 hours, the immature collateral vessels had returned to baseline reactivity.

Nitroglycerin improves indices of coronary collateral function when the vessels are well developed (Fam and McGregor, 1964; Cohen et al., 1973; Capurro et al., 1977). The literature remains controversial concerning the effect of nitroglycerin on collateral vessels in the innate, unstimulated state (Cohen et al., 1973; Bache et al., 1975; Becker, 1976). When the amount of blood flow to an ischemic area of myocardium is maintained at a constant level, the effect of nitroglycerin is to redistribute flow toward the endocardial layer (Swain et al., 1979). The data from the present study indicate significant redistribution of flow, as well as a significant increase in flow to layers 2, 3, and 4 in the collateral region during occlusion following nitroglycerin administration.

The nitroglycerin data, viewed independently, would imply a marked effect of nitroglycerin on the blood flow to a region of myocardium supplied by immature collateral vessels. These results, however, must be interpreted within the context of the prior occlusion, reperfusion, and reocclusion data. When compared with increases in collateral blood flow due to repeated occlusion alone, the increases due to nitroglycerin become less dramatic: in fact, the increase in mean flow was not statistically significant. Thus, investigations which utilize repeated occlusions to test the effects of drugs or other interventions on blood flow to an area of myocardium supplied by immature collateral vessels must be viewed within the context of these data.

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References


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