The Changing Anatomic Reference Base of Evolving Myocardial Infarction

Underestimation of Myocardial Collateral Blood Flow and Overestimation of Experimental Anatomic Infarct Size Due to Tissue Edema, Hemorrhage and Acute Inflammation

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SUMMARY The microsphere technique has been used to follow changes in collateral blood flow as a function of time and to relate acute changes in collateral flow to subsequent myocardial necrosis after coronary occlusion. However, flow studies in chronic preparations are complicated by an apparent loss of microspheres from necrotic myocardium. Preocclusion or control flow measured 2–4 days after coronary occlusion is reduced in infarcted compared with nonischemic areas. This apparent reduction in flow could result either from actual loss of microspheres from the infarct or from increased tissue weight. The latter possibility was examined by evaluating tissue components that could cause infarct expansion, i.e., water content and exogenous cellular elements (hemorrhage and inflammation), in 4- and 28-day-old infarcts produced by ligation of the circumflex artery in dogs. Infarct size measured at 4 days averaged 41% of the left ventricle (LV). Preocclusion blood flow (in ml/min/g) measured at 4 days was variably reduced in the infarcted circumflex (LCC) bed compared with the anterior descending (LAD) bed. Preocclusion LCC/LAD flow ratios ranged from 0.43–0.94. Analysis of these infarcts showed a 25% increase in infarct water content and extensive hemorrhage or acute inflammation or both, which added cellular elements to the infarcts; the severity of the latter was inversely proportional to the reduction in the LCC/LAD flow ratio. Thus, the decreased LCC/LAD ratios could be explained largely, if not entirely, by infarct expansion due to the addition of edema, hemorrhage and inflammation. By contrast, 28 days after coronary ligation, necrotic myocardium was largely replaced by dense scar tissue occupying only 11% of the LV. Contraction of these infarcts was associated with concentration of microspheres such that the LCC/LAD ratio was increased to 3.39. Thus, the changing composition of an evolving infarct results in a changing reference base. Early expansion of infarct volume due to edema and cellular infiltration causes an underestimation of collateral blood flow and an overestimation of infarct size when the data are referred either to tissue wet or dry weight. Later, when the infarct has been replaced by a contracted scar, directionally opposite errors occur. More accurate measurements of anatomic parameters or collateral flow can be achieved by using the preocclusion LCC/LAD flow ratio, as an index of the change in reference base, to calculate corrected anatomic and flow data.

TO ASSESS THE TIME COURSE of ischemic cell death and therapeutic interventions designed to limit infarct size, we developed an experimental model in which infarct size can be quantitated histologically.1 Myocardial necrosis is produced in open-chest dogs by temporary or permanent circumflex coronary (LCC) occlusions. Regional coronary blood flow is estimated with 7-10-μ microspheres at various times before and after coronary occlusion. Necrosis, the primary endpoint, is quantitated from representative histologic sections, and the resultant anatomic infarct size is evaluated within a framework of variables known to affect this process, including: 1) the amount of myocardium supplied by the LCC artery (determined by postmortem coronary dye injections),2 2) the collateral flow within that bed, and 3) hemodynamic parameters that alter the balance between myocardial oxygen supply and metabolic demand (heart rate, ventricular and peripheral pressures and contractility).

During these studies, we observed two unexpected results. First, preocclusion coronary flow in the LCC bed, quantitated from tissue samples obtained 4 days after the onset of infarction, was always less than preocclusion flow in the LAD bed. Second, the distribution of circumflex bed sizes was significantly larger in dogs with infarcts than in normal dogs. Many studies have confirmed that normal coronary arterial flow per gram of left ventricle is identical in the LAD and LCC beds.3,5 Therefore, we sought an explanation for the decreased LCC bed flow. Loss of microspheres from the infarct could account for the decrease.6,7 Alternatively, an increase in the volume of the infarcted circumflex bed due to tissue edema and/or increase in cellular elements within the infarct could account for the decrease in LCC control flow. We quantitatively assessed tissue edema and semiquantitatively assessed hemorrhage and inflammation in relation to apparent microsphere loss. Acute infarct volume was variably increased by a combination of tissue edema and hemorrhage or acute inflammation or both. Thus, the necrotic tissue had been replaced by scar tissue,
the infarct was markedly condensed and concentration of microspheres resulted in overestimation of flow. These parameters can be corrected from the measured preclosure LCC/LAD flow ratio.

Materials and Methods

Twenty-three healthy mongrel dogs that weighed 13.5-15 kg were studied. They were anesthetized with pentobarbital (30 mg/kg) and ventilated on a Harvard respirator with room air and enough oxygen to maintain physiologic blood gas and acid-base status. Clean surgical technique was used. Catheters were placed in the right femoral artery and vein. A small thoracotomy was performed in the fourth left interspace, and the pericardium was opened and sutured to the chest wall to create a temporary pericardial cradle. The circumflex artery was isolated beneath the left atrial appendage and a #1 silk suture placed around it. Catheters were placed, via the left atrial appendage, in the left atrium and left ventricle. The pericardial cradle then was relaxed. The ECG and left ventricular and peripheral blood pressures were monitored on a Brush 440 recorder.

Ten to 20 minutes after the preparatory surgery, the regional distribution of myocardial flow was measured by injecting 2-3 million microspheres through the left atrial catheter. An arterial blood reference sample was collected from the femoral artery at a rate of 7.8 ml/min beginning 15 seconds before and continuing for 2.5 minutes after the microsphere injection. The circumflex artery was permanently ligated 2-5 minutes later. Additional microsphere injections were made 20 minutes and 3 hours after coronary ligation, but these data are not included in the present report. The incisions were closed and the dogs allowed to recover for 4 or 28 days.

Fifteen of 19 dogs (79%) survived 4 days of LCC ligation, but one was excluded due to technical problems with the microsphere technique and another due to absence of severe ischemia after LCC occlusion. Three of four additional dogs (75%) survived 28 days after coronary ligation. The surviving dogs included in this report were reanesthetized, heparinized, and their hearts excised. The myocardium supplied by the left main and occluded circumflex arteries was identified by simultaneously perfusing the left main and circumflex artery distal to the ligature, with pink (1%) and blue (0.5%) monastral dyes (Dupont), respectively, in normal saline containing 6% dextran 70. Perfusion pressure was 100-120 mm Hg. Each heart then was fixed for 48 hours (minimum) in 10% formalin. The hearts were weighed before and after perfusion and fixation.

The formalin-fixed left ventricles (including the septum) were dissected free and cut into nine transverse slices of approximately equal thickness (fig. 1). The size of the LCC bed (as a percentage of the left ventricle) was determined morphometrically from color photographs of these nine slices. Five of these slices were used for quantitative estimation of infarct size. Ischemic and nonischemic regions of the other four slices were subdivided into inner, middle, and outer thirds for counting and determination of flow. The lateral junctions between nonischemic and ischemic regions were excluded in order to prevent cross-contamination of samples.

The samples used for microsphere counting from four dogs with 96-hour infarcts were subsequently dried overnight at 105°C and tissue water was calculated as a percentage of wet weight and as milliliters of H₂O/g dry weight. Flows were calculated based on tissue wet weight as well as tissue dry weight. Histologic sections from slices two and five (corresponding to the levels used for flow analysis) were examined for qualitative changes in the evolving infarcts. Cellular elements that could increase tissue weight, i.e., hemorrhage and inflammatory cells, were assessed on a semiquantitative basis from 0-4+. Beginning organization (macrophage phagocytosis of necrotic fibers), which might either increase or decrease tissue weight, was assessed similarly. Histologic sections were graded without knowledge of the relative flow reduction.

All quantitative results are expressed as group mean ± SEM.
Four-day Infarcts

Regional blood flow, measured before coronary occlusion, averaged $1.31 \pm 0.11$ ml/min/g wet weight in the LAD bed of the 13 dogs with 4-day infarcts (table 1). This flow rate was equivalent to control flows measured in acute experiments using the same animal preparation and postmortem techniques. Control LAD flow in 11 dogs sacrificed acutely was $1.18 \pm 0.09$ ml/min/g (Reimer KA, Jennings RB: unpublished observations). Thus, there was no apparent loss of microspheres from nonischemic myocardium during the first 4 days of myocardial infarction. However, preocclusion blood flow in the infarcted circumflex arterial bed was significantly less than the preocclusion flow to the nonischemic LAD bed. The LCC/LAD ratios were always less than 1.0 and ranged from 0.43–0.94 (tables 1 and 2).

Postmortem coronary injection and subsequent formalin fixation caused relatively slight changes in overall cardiac weight (table 1). Although approximately 150 ml of dye solutions were used to perfuse each heart, virtually all of this fluid drained from the heart during perfusion by way of the coronary sinus. Heart weights after perfusion were increased by 1–4 g. The prevention of cardiac edema was facilitated by the use of dextran as the suspending medium. (In pilot studies, saline infusion caused a significant increase in cardiac weight.) Formalin fixation usually caused a slight dehydration and the final heart weights averaged 2 g (2%) less than initial weights. The lack of a significant effect of coronary perfusion and formalin fixation on heart weight is reflected by the normal tissue water content in nonischemic myocardium (table 2).  

The low circumflex flows could be related, in part, to edema within the circumflex bed. On the average, the circumflex bed water content increased from 3.86 to 4.82 ml/g dry weight, an increase of 25%. The largest increase was 39%, observed in dog no. 4, which had the greatest reduction in LCC bed flow. This increased water content was not restricted to subendocardial or subepicardial layers, but occurred throughout the circumflex bed.

Recalculation of flows based on dry rather than wet weights in four dogs (table 2) only partially corrected the LCC/LAD ratio from 0.66 to 0.78, compared with the predicted ratio of 1.0. Thus, edema accounted for only one-third of the average apparent microsphere loss. In order to evaluate whether infarcts were significantly swollen by hemorrhage and inflammation as well as by edema fluid, these features were analyzed on histologic sections of the infarcts.

All 4-day-old infarcts had the general zonal characteristics described previously (figs. 2–4). There was a central core of necrotic myocardium devoid of both hemorrhage and inflammation. This core was located primarily within the subendocardial half of the infarct and was presumably caused by microvascular necrosis, with essentially complete loss of perfusion.
Peripheral to this central core was a zone of hemorrhage and acute inflammation and peripheral to this, a subepicardial zone of beginning repair. However, these zones had irregular and often patchy distributions and were never confined to one radial location (inner, middle, or outer third) within the infarct.

Although there was some hemorrhage and inflammation in all infarcts, the relative amounts varied considerably among dogs (figs. 2, 3 and 5). This variation was evaluated on a semiquantitative 0-4+ grading system without knowledge of the associated LCC/LAD ratios (table 1). The histologic findings correlated well with the reduction in LCC/LAD flow ratio. The dog with the highest flow ratio (dog no. 2) had minimal hemorrhage and virtually no acute inflammation. (fig. 5). The dog with the second highest flow ratio (dog no. 12) also had minimal hemorrhage and inflammation. In contrast, the dog with the lowest flow ratio (dog no. 4) had massive and widespread hemorrhage and broad zones of polymorphonuclear infiltration (fig. 3). The remaining dogs had intermediate flow ratios and moderate amounts of hemorrhage or acute inflammation or both.

Thus, histologic evaluation indicated that much of the apparent microsphere loss not accounted for by edema could be explained by the presence of hemorrhage or acute inflammation or both in the infarcts. Although the amount of extraneous cellular elements adding dry weight to the infarct was not precisely quantitated, pronounced hemorrhage or inflammation or both were associated with low LCC/LAD flow ratios.

Organization, consisting of macrophage infiltration, phagocytosis of necrotic myocardium and fibroblastic proliferation (figs. 2, 3 and 5), had begun on the periphery of all infarcts, but was not extensive in any case. Whether this early organization might lead to increased (addition of macrophages and fibroblasts) or decreased (removal of dead cells) tissue weight is unknown.

**Twenty-eight-day Infarcts**

After 28 days, most of the necrotic muscle had been replaced by scar tissue composed largely of fibroblasts in a dense collagen matrix (fig. 4). However, small subendocardial islands of organizing necrosis were still present in all three infarcts and there were small foci of calcified myocytes in the subepicardial zone.

Infarct size at 28 days ranged from 6-16% of the left ventricle (table 3), indicating that these scars were markedly condensed compared with 4-day infarcts. The extent of both circumferential and base-apex involvement was reduced, and the posterior wall was thinner than the noninfarcted anterior wall, indicating a three-dimensional contraction of these scars.

The LCC/LAD flow ratio measured before LCC occlusion was greater than 1.0 in all three of these dogs and averaged 3.39 (table 3). Thus, in contrast to
Figure 2. The characteristic zonal features of a 4-day-old infarct in dog no. 3. The view is from the center of the circumflex bed and incorporates much of the transmural wall from the subendocardial region (top) to subepicardial region (bottom). There is a subendocardial central core of coagulation necrosis (N), with separation of fibers due to interstitial and cellular edema, but with relatively little cellular infiltration. This zone is surrounded by a zone with hemorrhage (H) and an intense acute inflammatory response (I). In the peripheral zone, organization (O) has begun and is characterized by macrophages, which have removed some of the necrotic cells, and ingrowth of fibroblasts and capillaries. This is better seen in the lower inset. Some heavily calcified cells (Ca) also are present in the peripheral zone. Some viable muscle (V) survived in the subepicardial region seen at the lower right. This region between the organizing zone of the infarct and viable myocardium is shown at higher power in the upper inset. Stained with hematoxylin and eosin, magnification X 55; insets X 120.
Figure 3. The infarct with the greatest "microsphere loss" (dog no. 4) is shown. The view encompasses much of the transmural dimension of this infarct from the subendocardial region (top) to subepicardial region (bottom). A core of coagulation necrosis (N) with little cellular reaction is surrounded by areas with massive hemorrhage (H) and interstitial inflammation (I). The subendocardial region, seen better in the upper inset, contains necrotic muscle widely separated by large collections of extravasated erythrocytes, intermixed in some places with polymorphonuclear neutrophils. In midmyocardial layers of this infarct (lower inset), necrotic muscle fibers are separated primarily by a massive infiltration with degenerating polymorphonuclear neutrophils. Hemorrhage and inflammation could account for well over half of the cellular components of this region of this infarct, and overall tissue weight would be correspondingly increased. Organization (O), similar to that seen in figure 2, has begun in the peripheral zone of this infarct. Stained with hematoxylin and eosin; magnification × 55; insets × 250.
FIGURE 4. Histologic sections from representative 4-day-old (dog no. 13) and 28-day-old (dog no. 2) infarcts. Both illustrations show transmural sections of the core of the infarct from slice 5 (see figure 1). The boundaries between infarct and viable myocardium are marked by solid lines. The 4-day-old infarct (top) shows the zonal characteristics described previously. There is a subendocardial core of coagulation necrosis (N) surrounded by an irregular zone of necrotic muscle swollen by hemorrhage (H) and inflammation, and an outer zone of necrotic muscle beginning to organize (O). Viable muscle (V) is present in the subepicardial zone. At 28 days (bottom), focal subendocardial necrosis (N) is still present, but most of the infarct has been replaced by a fibrotic scar (F). The two hearts were equivalent in size (123 g and 134 g) and are shown at the same magnification. Contraction of the 28-day-old scar has markedly reduced the apparent size of the original infarct in both the transmural and circumferential dimensions. Expansion of the 4-day-old infarct by hemorrhage and inflammation and contraction of the 28-day-old scar caused dilution and concentration, respectively, of microspheres injected before coronary ligation. Magnification × 6.

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<th>Table 3. Gross, Histologic and Flow Analyses of 28-day-old Infarcts</th>
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Abbreviations: LAD = left anterior descending artery; LCC = circumflex artery; LV = left ventricle.
The infarct with the least "microsphere loss" (dog no. 2) is shown. The view includes midmyocardial (top) and epicardial (bottom) regions. Although there is hyperemia (H) (and/or hemorrhage) and an acute inflammatory infiltrate between necrotic muscle fibers (better seen in the inset), the massive collections of red and white cells shown in figures 2 and 3 were not observed. The small amount of hemorrhage and inflammation in this infarct was independent of the extent of necrosis. As in other 96-hour infarcts in this study, necrosis was almost transmural except for scattered subepicardial islands of viable (V) muscle cells. Organization (O) similar to that in figures 2 and 3 had begun in the peripheral region of infarct. Stained with hematoxylin and eosin; magnification × 100; inset × 300.

Discussion

The Changing Anatomic Reference Base of Evolving Infarction

The microsphere technique has been used to follow changes in collateral blood flow as a function of time
and to relate acute changes in collateral flow to subsequent myocardial necrosis after coronary occlusion.\textsuperscript{10-15} In hearts with 4-day-old infarcts, preocclusion blood flow based on formalin-fixed tissue wet weight was reduced in infarcted compared with nonischemic myocardium of the same animals. This finding prompted us to reevaluate the reference base used for anatomic or flow studies in evolving myocardial infarcts. As an infarct develops, the myocardium first becomes edematous. Later, hyperemia, hemorrhage and inflammation appear and are followed by the process of repair. Thus, an evolving infarct goes through a series of inflammatory and healing stages with eventual replacement of the dead myocardium by collagenized scar tissue. An infarct at 24 or 96 hours or at later stages of repair is no longer the same as an infarct at 2 hours. It has been generally accepted from pathologic observations of myocardial infarcts of various ages that the final scar occupies a volume much smaller than the myocardium that it replaces. Although the problem of the changing infarct composition has been noted,\textsuperscript{16} early changes in infarct volume have not been evaluated in detail. It is an intuitive deduction that myocardium to which edema, hemorrhage and inflammatory cells have been added must have an increased volume, whereas myocardium from which myofibrillar protein and myocytes have been removed should have a decreased volume. Consequently, neither tissue wet weight nor dry weight are totally valid reference bases to which microsphere or anatomic measurements can be referred.

At 4 days, infarct volume and weight were increased because of both edema and the addition of cellular elements, i.e., hemorrhage and inflammation. This increased infarct volume was associated with artifactually low preocclusion flows in the infarct. Tissue edema increased infarct volume by 25\% and accounted for a significant portion (about one-third on the average) of this error in flow. However, calculation of blood flow based on tissue dry weight eliminated the observed flow discrepancy only in the dog (no. 2) that had virtually no hemorrhage or inflammatory cell infiltration. In the other dogs, both wet and dry weights of the infarct were increased by hemorrhage and inflammatory cell infiltration. The amount of hemorrhage and/or inflammation varied, but was inversely proportional to the observed LCC/LAD flow ratio. This correlation supports the conclusion that cellular elements as well as edema contributed to the increased volume of the infarcts and the low infarct flows that resulted.

A similar preocclusion flow reduction has been observed by others\textsuperscript{6,7} in 1-4-day-old infarcts, but has been interpreted, without evidence, to indicate that microspheres are washed out of necrotic myocardium. We cannot exclude the possibility that a few microspheres could gradually be washed out of either necrotic or normal myocardium. However, the marked apparent gain of microspheres that occurred in the 28-day infarcts in which the swollen necrotic muscle had been replaced by dense scar tissue indicates that selective microsphere loss from the infarct was minimal. Thus, the results of the present study rule out loss of significant numbers of microspheres from necrotic myocardium. Rather, myocardial infarct volume is a changing reference base that introduces systematic errors into measurements of flow and anatomic parameters.

We obtained our results in coronary perfused, formalin-fixed hearts, and the differences in infarct vs viable tissue water content might be influenced by these techniques. The relevant issue, however, is not the source of the edema fluid, but rather, under the conditions of analysis, that differences in the water content of infarcted vs control myocardium contributed to error in estimating collateral flow.

The extent of infarct edema and consequent erroneous flow estimation was not evaluated in fresh tissue. However, the results would be similar because the tissue waters of the fixed hearts were similar to those previously observed in fresh tissue. Despite perfusion and fixation, the water content of nonischemic myocardium was similar to that of fresh heart muscle,\textsuperscript{8} and the 25\% increase in the water content of the infarcted region was consistent with the edema reported in unperfused, unfixed infarcts.\textsuperscript{8,16} In addition, tissue hemorrhage and inflammation cannot be artifactually induced. Thus, it is likely that conclusions derived from this study with perfused, fixed hearts are valid for fresh tissue as well.

**Effect of Infarct on Estimates of Ischemic Bed Size and Infarct Size**

An increase or decrease in tissue weight would cause significant errors, not only in the measurement of blood flow, but also in the estimation of anatomic parameters calculated in infarct sizing studies. In our studies, infarct size (as a percentage of the left ventricle) is evaluated relative to the myocardium at risk, i.e., the area normally perfused by the circumflex artery.\textsuperscript{1,2} An increase in infarct volume would cause overestimates of both the size of the circumflex bed and the size of the infarct that develops within that bed.

Overestimation of anatomic parameters occurs in 4-day-old infarcts because of tissue expansion (fig. 6). The volume of myocardium supplied by the LCC artery in the 13 dogs with 4-day-old infarcts described in the present study is compared with that in 11 dogs (Reimer KA, Jennings RB: unpublished data) without infarcts. The latter dogs had acute coronary occlusions but were sacrificed on the day of study. The postmortem techniques of coronary perfusion and evaluation of LCC bed size were identical to those used in the present study. In the series of 11 hearts studied acutely, the circumflex bed ranged from 30-43\% of the left ventricle (mean 37 \pm 1.1\%). In contrast, the size of the circumflex bed in dogs with 4-day-old infarcts after LCC ligation ranged from 42-66\% of the left ventricle (mean 51 \pm 2.0\%; \( p < 0.001 \)). The LCC/LAD flow ratio in each dog was used to correct for the increased tissue weight in the LCC bed and to calculate a corrected circumflex bed size.
rected circumflex bed \((\text{CB}_c)\) as a percentage of the left ventricle is:

\[
\text{CB}_c = \frac{\text{CB} \cdot \frac{\text{LCC}}{\text{LAD}}}{\text{CB} \cdot \frac{\text{LCC}}{\text{LAD}}} + (100 - \text{CB})
\]

where \(\text{CB}\) and 100 – \(\text{CB}\) are the uncorrected LCC and nonischemic beds as percentages of the left ventricle. This correction brought the LCC bed size of dogs with 4-day-old infarcts into the normal range (table 1 and fig. 6).

Because of the development of extensive collateral channels in hearts with 28-day-old infarcts, coronary perfusion studies were inadequate for the identification of LCC and LAD arterial beds in these hearts. However, the sizes of the LCC beds, as estimated from the lateral margins of the scar, were much smaller than the LCC beds in hearts with 4-day-old infarcts (table 3). The equation derived above evidently overcorrected ischemic bed size in these dogs. This overcorrection may have occurred because by 28 days, not only were the infarcts condensed, but the nonischemic areas were increased by cardiac muscle hypertrophy. Thus, the LCC/LAD ratio would have been increased both by microsphere concentration in the infarct and microsphere dilution in the nonischemic region. If so, the LCC/LAD ratio would have overestimated infarct condensation. Whenever the nonischemic reference base changes for any reason, e.g., myocardial hypertrophy, the LCC/LAD ratio would not provide an accurate correction factor for ischemic bed size.

Infarct size as a percentage of the left ventricle can be corrected during the acute phase of infarction by an equation equivalent to that shown above. These corrected infarct sizes (table 1) should more accurately reflect the amount of muscle that originally underwent coagulation necrosis. Again, however, corrections made for healed infarcts (table 3) will be inaccurate to the extent that hypertrophy or other changes have occurred in nonischemic muscle.

In summary, in the early stages of myocardial infarction, the infarcted region gains weight both by the addition of edema fluid and cellular elements, i.e., hemorrhage and acute inflammation. This weight gain causes a variable underestimation of blood flow (using the microsphere technique) and overestimation of anatomic measurements of coronary bed size and infarct size. Failure to make appropriate corrections caused errors that varied from dog to dog, but were as large as 50% in 4-day-old infarcts. As necrotic muscle is replaced by scar tissue, there is a marked loss of infarct mass that causes overestimation of flow and underestimation of anatomic measurements. Errors of up to 400% occurred in 28-day-old infarcts. As long as nonischemic muscle has not changed, the preocclusion LCC/LAD blood flow ratio is an index of this tissue swelling or shrinkage and can be used as a correction factor to improve the accuracy of both flow and anatomic measurements.

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