Noninvasive Prediction of Ultimate Infarct Size at the Time of Acute Coronary Occlusion Based on the Extent and Magnitude of Collateral-Derived Myocardial Blood Flow

Matthew P. Coggins, MD; Jiri Sklenar, PhD; D. Elizabeth Le, MD; Kevin Wei, MD; Jonathan R. Lindner, MD; Sanjiv Kaul, MD

Background—We hypothesized that by detecting regions with adequate collateral-derived myocardial blood flow (MBF) within the risk area (RA), we could predict ultimate infarct size (IS) at the time of coronary occlusion.

Methods and Results—Group 1 dogs (n=15) underwent coronary occlusion without reperfusion, whereas group 2 dogs (n=6) underwent both occlusion and reperfusion. RA was measured with aortic root injections of microbubbles. Myocardial contrast echocardiography (MCE) was performed with high mechanical index intermittent harmonic imaging at pulsing intervals (PIs) of <1 to 30 cardiac cycles during an intravenous infusion of microbubbles (Sonozoid). MBF was measured with radiolabeled microspheres, and postmortem tissue staining was used to determine IS. Perfusion defect size (PDS) on MCE varied with the PI and was largest at a PI of 2.6±0.4 seconds, where it correlated well with RA (r=0.82). PDS was smallest at a PI of ≥10.6±1.5 seconds, where it correlated closely with IS (r=0.92). Areas that underwent necrosis could be identified early after coronary occlusion as having the lowest microvascular flow velocity (V) and MCE-derived MBF (A×V). The results were similar with or without reperfusion. Because of variability in collateral-derived MBF, there was no correlation between RA and ultimate IS (P=0.37). The extent of regional dysfunction also correlated poorly with IS (r=0.31).

Conclusions—MCE can be used immediately after coronary occlusion to define ultimate IS by measuring the magnitude and spatial extent of collateral-derived residual MBF within the RA. Thus, it could help individualize risk and management in acute myocardial infarction. (Circulation. 2001;104:2471-2477.)

Key Words: collateral circulation • infarction • contrast media

For a given duration of coronary occlusion, the size of the risk area (RA) and the amount of residual collateral-derived myocardial blood flow (MBF) within the RA are the major determinants of ultimate infarct size (IS).1–3 Similarly, for a given RA size and duration of coronary occlusion, IS depends on the magnitude and spatial extent of residual collateral-derived MBF.1–3

Currently, IS can be measured by myocardial contrast echocardiography (MCE) only after reperfusion, by exploiting the “no-reflow” state of necrotic tissue.4–8 In this setting, fewer microbubbles enter the necrotic zone because of functional and/or structural damage to the capillaries. Because of reactive hyperemia after successful reperfusion, IS can be underestimated by MCE. In this setting, exogenous hyperemia can unmask abnormal microvascular reserve within the necrotic area and provide a more accurate assessment of IS.4–6 In the presence of persistent coronary occlusion, however, there is no in vivo technique that can predict ultimate IS at the time of coronary occlusion. This information could be valuable, because a priori determination of ultimate IS could assist in risk stratification and management of acute myocardial infarction (AMI).

We can measure regional MBF using MCE, in which we destroy microbubbles within the myocardium during their steady-state intravenous infusion and then measure their rate of myocardial replenishment.9 We therefore hypothesized that if enough time is allowed after microbubble destruction, we can define regions within the RA in which their replenishment is slow because of low levels of MBF emanating from collaterals. It is known that myocytes can remain viable at as little as 25% of normal MBF.1–3 Thus, the spatial distribution of microbubbles within regions exceeding this level could be used to identify tissue with the potential to be spared from necrosis even during persistent coronary occlusion. We have previously shown that regions with adequate collateral flow during coronary occlusion are those that are spared necrosis after reperfusion.10 Therefore, this method could predict IS at the time...
of coronary occlusion even if reperfusion were achieved later.

Methods

Animal Preparation

The protocol was approved by the Animal Research Committee at the University of Virginia and conformed to the American Heart Association “Guidelines for Use of Animals in Research.” Twenty-one open-chest anesthetized dogs were used for the experiments. Catheters were placed in both femoral veins for administration of fluids and infusion of microbubbles, as well as in both femoral arteries for withdrawal of duplicate arterial blood samples for radiolabeled microsphere MBF analysis. Catheters were also placed in the ascending aorta for measurement of pressure and for bolus administration of microbubbles to define RA, in the left atrium for injection of radiolabeled microspheres, and in the right atrium for measurement of right atrial pressure. The proximal portions of the left anterior descending (LAD) and left circumflex (LCx) coronary arteries were dissected free from surrounding tissues. Flow probes were placed around both vessels and connected to a flowmeter.

Myocardial Contrast Echocardiography

High mechanical index (0.9) intermittent harmonic imaging was performed in the parasternal short-axis plane at the mid papillary muscle level with the Sonos-5500 system (Agilent-Philips). The transducer was fixed in position distal to the site of occlusion, and a water bath placed over the heart served as an acoustic interface. A dynamic range of 60 dB was used, and the depth, focal point, and gain were optimized at the beginning of each experiment and were held constant throughout.

RA was defined with bolus injections of 2 mL of Albunex (Mallinkrodt Medical) administered into the aortic root. A suspension of 1 mL of Sonozoid (Nycemed Amersham) in 49 mL of normal saline was used as the venous contrast agent. It is a perfluorocarbon-containing lipid microbubble with a mean diameter of 3 μm and a mean concentration of 10⁹ microbubbles/mL. The infusion rate (1 to 2 mL/min) for each dog was adjusted to provide optimal myocardial opacification with minimal left ventricular (LV) posterior wall shadowing.

End-systolic images were obtained at pulsing intervals (PIs) ranging from <1 to up to 30 cardiac cycles. Intervals of <1 cardiac cycle were achieved by use of dual pulses at end systole. Five end-systolic images acquired at a PI of 1 cardiac cycle before microbubble administration served as background images. Five images were then acquired at each PI during microbubble infusion.

Because of postprocessing and log compression, the relation between backscatter and video-intensity (VI) measurements is not linear. To linearize the data, VI values within every pixel in each image underwent log-linear transformation by use of lookup tables provided to us by the manufacturer of the system. Using in vitro studies, we confirmed that the VI values measured after this transformation are very similar to those measured by acoustic densitometry, which is a measurement made from digital data before log compression and postprocessing.

Images at each PI were aligned by use of computer cross-correlation. Regions of interest were placed over the normal myocardium and within different parts of the RA (endocardial and epicardial halves of the mid and later thirds), and VI was automatically measured from these regions from each of the aligned images. PI versus background-subtracted VI plots were then generated and were fitted to the exponential function $y = A(1 - e^{-t})$, where $y$ is VI at a PI of $t$, $A$ is the plateau VI representing capillary blood volume, and $β$ represents the mean microbubble velocity. The product $A/β$ denotes MCE-derived MBF.

In addition to the above analyses, color coding was applied to background-subtracted images to visually enhance regional differences in myocardial contrast enhancement as previously described. Perfusion defect size (PDS) was planimetered at each PI from this set of images by a blinded observer. RA was measured from the gray-scale images obtained during aortic root injections of Albunex.

Wall Thickening Analysis

The short-axis slice corresponding to the MCE image was analyzed along 100 circumferentially placed chords between the epicardial and endocardial outlines in a representative systolic contraction sequence (from end diastole to end systole). Wall thickening (WT) in the central 50% of the nonoccluded bed (defined by MCE) was measured, and the mean ± 1 SD of the values within the region was derived. Any chord demonstrating less than the mean −2 SD of WT in the normal bed was classified as abnormal. The percentage of the LV short-axis slice demonstrating chords with abnormal WT was then calculated. In this manner, both the circumferential extent and the degree of abnormal WT were defined.

Experimental Protocol

After baseline data had been obtained, either the LAD ($n=11$) or the LCx ($n=10$) was occluded. Group 1 dogs ($n=15$) underwent 6 hours of coronary occlusion without reperfusion, whereas group 2 dogs ($n=6$) underwent 2 to 6 hours of coronary occlusion followed by 30 to 165 minutes of reperfusion. As soon as hemodynamics had stabilized after coronary occlusion (~30 minutes), an aortic root injection of Albunex was performed in 10 group 1 dogs to define RA. MCE was performed with a continuous venous infusion of Sonozoid. MCE and hemodynamic measurements were repeated at 2, 4, and 6 hours after coronary occlusion in group 1 dogs and just before reperfusion in group 2 dogs. Radiolabeled microspheres were injected at 30 minutes and 6 hours after coronary occlusion in the group 1 dogs and just before reperfusion in the group 2 dogs. After 30 minutes of reperfusion, the group 2 dogs underwent MCE during coronary hyperemia. At the end of the experiment, a needle was passed through the heart at the level of the transducer to identify the MCE image slice, and the dogs were euthanized. The heart was removed and sliced for measurement of radiolabeled microsphere-derived MBF and IS.

Statistical Methods

Data are expressed as mean ± 1 SD. Stages and defect sizes at different PIs were compared by repeated-measures ANOVA. Correlations between RA and PDS, as well as between IS and PDS, were performed by least-squares fit regression analysis. Differences were considered significant at a value of $P<0.05$ (2-sided).

Results

Group 1 Dogs

The hemodynamics remained stable during the entire occlusion period, and there were no differences in transmural, endocardial, or epicardial MBF at 30 minutes and 6 hours after coronary occlusion (Table 1).

RA Versus PDS

Figure 1A shows an example of RA using aortic root injection of Albunex, and Figure 1B to 1D demonstrates myocardial perfusion during Sonozoid infusion at PIs of 0.5, 2.5, and 10 seconds. PDS at a PI of 2.6±0.4 seconds, when replenishment of normally perfused myocardium is almost complete, correlated best with RA ($r=0.82, P<0.01$). At longer PIs, progressively greater opacification was noted within the RA, which represented collateral-derived MBF. Hence, the PDS at longer PIs underestimated the RA and correlated poorly with it ($r≤0.52$). The PDS at each PI remained unchanged during 6 hours of coronary occlusion (Table 1).
IS Versus PDS

PDS at different PIs had a relation with IS that was opposite to the relation with RA. As the PI was lengthened, the relation between PDS and IS improved. Figure 2 depicts the relations between IS and PDS at 4 different PIs in all group 1 dogs. At a PI of 2.6 seconds, there was no correlation between PDS and IS. The PDS at this PI was approximately the same in all dogs, but IS varied widely. PDS at a PI at 5.3 seconds had a better relation with IS but overestimated it. PDS at a PI of 10.6 seconds predicted IS most accurately. The correlation between PDS at long PIs and IS was similar for both the LAD (r = 0.84) and LCx (r = 0.87) beds.

Images acquired at longer PIs demonstrated opacification of regions within the RA receiving MBF via collaterals. As long as the MBF level responsible for this delayed opacification was sufficient to maintain myocardial viability, the PDS represented the extent of tissue still at risk for necrosis.

There was no difference in PDS at 20 versus 30 cardiac cycles. Therefore, a region within the RA that did not demonstrate opacification by 20 cardiac cycles (mean of 10.6 seconds), did not show opacification at a later time.

Infarcts ranging in size from negligible to nearly transmural from 3 different group 1 dogs are depicted in panel D of Figure 1.

**Figure 1.** RA during aortic root injection of Albunex (A) and PDS during continuous infusion of Sonozoid at increasing PIs in a group 1 dog. Myocardial opacification is only partial at a PI of 1.5 seconds (B). PDS at a PI of 2.5 seconds, however, appears almost identical to size of RA (C). At a PI of 9.9 seconds, there is no longer a perfusion defect because of collateral-derived MBF (D). There was no infarction in this dog despite a large RA and 6 hours of total coronary occlusion.

**Figure 2.** Correlations between IS and PDS during continuous infusion of microbubbles at various PIs in all group 1 dogs. Correlation is best at a PI > 10.6 seconds. See text for details.
Figures 3 to 5. Whereas the PDS at the shortest PI (representing RA as discussed above) was very similar for all 3 dogs (panel A), the PDSs at the longest PI (10.6/11006 1.5 seconds) were very different from one another (panel C). At this PI, all regions within the ultrasound imaging plane with adequate MBF (normal myocardium through nonoccluded coronary arteries or ischemic myocardium through collateral vessels) were replenished with microbubbles, and only regions with very low MBF did not receive microbubbles. The regions without opacification closely resembled the topography of necrosis (panel D).

MBF Versus IS
Figure 6 illustrates PI versus VI curves from several regions within the RA from 1 group 1 dog ranging from those with the lowest MBF (endocardium in the central one third) to the highest MBF (lateral aspects and central one third of the epicardium). A curve from the adjacent normal myocardium is also shown. It is apparent that both A and β values increased with higher MBF. Consequently, the product A×β, which represents total regional MBF, was a predictor for regions that did not undergo necrosis.

Table 2 shows mean values of A, β, and A×β for regions within the RA with mild, moderate, or severe reductions in MBF. Although A was significantly reduced at all MBF levels, both mild (>50% of baseline) and moderate (25% to 50% of baseline) reductions in MBF resulted in similar values for A, whereas regions with severe reductions in MBF, which were associated with necrosis, showed a significantly greater reduction in A. Because “no reflow” is not expected at 30 minutes after coronary occlusion, the low values of A may simply mean that because of very low MBF, microbubbles had not yet reached these regions. In comparison, both β and A×β progressively decreased with greater reductions in MBF.

Regions that demonstrated initial opacification by 10.6 seconds had adequate MBF to maintain tissue viability (mean of 29% compared with normal myocardium). In contradistinction, regions that did not exhibit any myocardial opacification by this time were likely to undergo necrosis. In 90% of these regions ultimately undergoing necrosis, MCE-derived MBF (A×β) was <20% of normal, and radiolabeled microsphere-derived MBF was <13% of normal.

Relation Between RA, IS, and Abnormal WT
The circumferential extent of abnormal WT correlated well with RA in the 10 group 1 dogs in which the latter was measured (r=0.72, P<0.02). Furthermore, the extent and degree of abnormal WT remained unchanged during the entire 6-hour occlusion period (Table 1). Because IS varied markedly between dogs with similar-sized RAs based on different amounts of collateral MBF, there was no relation between RA and IS (r=0.37, P=NS). The extent of abnormal WT markedly overestimated IS and correlated poorly with it (r=0.31, P=NS).

Group 2 Dogs
These dogs underwent various periods of coronary occlusion (282±122 minutes) to create infarcts of different sizes. The aim was to determine whether prediction of IS during coronary occlusion was the same as that seen after variable periods (282±122 minutes) of reperfusion (in the presence of...
vasodilation in the latter setting\textsuperscript{4–6}). PDS at 30 minutes after coronary occlusion was similar to that after 30 minutes of reperfusion (15.3\% ± 12.3\% versus 14.9\% ± 12.4\% of the LV short-axis slice) and correlated well with it (\(r = 0.90\)). PDS at both times were also similar to IS (13.2\% ± 11.5\% of the LV short-axis slice) and correlated well with it (\(r = 0.95\)). Thus, reperfusion did not have any effect on the IS predicted at 30 min after coronary occlusion.

### Discussion

**Determinants of IS**

After abrupt coronary occlusion, necrosis begins in the endocardium and then migrates transmurally over time.\textsuperscript{1,2} If there is very little or no residual collateral-derived MBF, most of the RA undergoes necrosis within 6 hours.\textsuperscript{2} In this setting, therefore, the duration of coronary occlusion is the ultimate determinant of the IS/RA ratio.

Another major determinant of the IS/RA ratio is the magnitude and extent of residual collateral-derived MBF within the RA.\textsuperscript{1,2} If MBF is \(>25\%\) of normal, myocytes can remain alive despite prolonged coronary occlusion\textsuperscript{1–3} and lack of function that occurs at this level of MBF.\textsuperscript{16,17} Thus, if regions with low MBF that is adequate to sustain myocardial viability can be defined within the RA at the time of coronary occlusion, then the ultimate “worst-case scenario” IS can be predicted early after onset of AMI.

Currently, myocardial perfusion imaging can be used to define RA on the basis of a sharp gradient in MBF between normal and underperfused myocardium.\textsuperscript{4–8} Because of large variability in collateral-derived residual MBF, IS can vary widely despite similar-sized RAs and duration of coronary occlusion.\textsuperscript{1,2} If the “worst-case scenario” ultimate IS can be determined a priori, then patients with AMI can be triaged on the basis of the potential IS rather than the size of RA.

IS imaging has been used clinically for almost 2 decades. Infarct-avid imaging agents such as \(^{99m}\text{Tc}\) pyrophosphate and \(^{123}\text{I}\)-labeled antimyosin antibodies can define the size of irreversible myocardial injury only after it has already occurred but cannot predict IS at the time of coronary occlusion.\textsuperscript{18,19} Similarly, IS can be defined by both MCE and single photon emission CT only after successful reperfusion.\textsuperscript{20–22} In this situation, IS is defined on the basis of “no reflow” within the infarct zone. In this study, we have shown that by defining the magnitude and spatial extent of collateral-dependent residual MBF within the RA, we can use MCE to predict ultimate IS at the onset of coronary occlusion. The prediction

### Table 2. MCE Parameters Versus Radiolabeled Microsphere–Derived MBF Within the RA of the Group 1 Dogs

<table>
<thead>
<tr>
<th>Variable, % of Normal</th>
<th>Mild Reduction in MBF ((&lt;=50% of Normal))</th>
<th>Moderate Reduction in MBF (25%–50% of Normal)</th>
<th>Severe Reduction in MBF ((&lt;25% of Normal))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>79 ± 42*</td>
<td>88 ± 35*</td>
<td>49 ± 45†</td>
</tr>
<tr>
<td>(\beta)</td>
<td>54 ± 23*</td>
<td>32 ± 12†</td>
<td>25 ± 20†</td>
</tr>
<tr>
<td>A(\times)(\beta)</td>
<td>38 ± 21*</td>
<td>26 ± 13†</td>
<td>9 ± 7†</td>
</tr>
</tbody>
</table>

*\(P<0.05\) vs normal bed.

†\(P<0.05\) vs regions with mild reduction of MBF.

‡\(P<0.05\) vs regions with moderate reduction of MBF.
of IS was independent of whether or not reperfusion was achieved as well as the duration of reperfusion.

Limitations of the Study
Like all imaging techniques, MCE also has artifacts, particularly in regions in which either the bubbles are destroyed in excess (such as the near field) or are not destroyed sufficiently (as at the 2 sides of the sector). These artifacts generally have a distinct appearance and can be discriminated from actual perfusion defects. Similarly, far-field shadowing from the presence of microbubbles in the LV can result in artifacts, as shown in Figures 4 and 5, that may also impair the ability to accurately measure PDS.

We defined PDS by planimetry. We did not define any threshold value for hypoperfusion. Part of the reason is the variability of VI values within different regions of the myocardium, which is dependent on the heterogeneity in the acoustic power within the ultrasound field. New low-frequency transducers transmitting defocused beams have reduced this heterogeneity, and it may be possible to define normal values for different myocardial regions in the future, thereby allowing PDS measurement based on objective criteria.

The method of obtaining long PIs may be difficult to achieve in the clinical setting. Acquisition methods have been developed, however, whereby the PIs are increased automatically to create cine loops that take no more than 30 seconds to create, which is not a long time to hold a transducer in a single position. In addition, “real-time” methods allow very rapid data acquisition without the need to hold the transducer steady for even that long. 3D imaging will also allow a more comprehensive examination of the heart within a shorter period of time.

We saw no difference in our prediction of ultimate IS whether or not we caused reperfusion. There is controversy as to whether reperfusion increases IS, part of which may be related to the animal model used. If there is enough collateral MBF within an RA, O$_2$ tension in that region should be adequate so that the levels of free radicals generated within that region after reperfusion are not high enough to cause further necrosis. This may be in contradistinction to models in which collateral flow is absent and O$_2$ tension in ischemic but nonnecrotic cells is very low. Reperfusion in this situation could result in high levels of free radicals that may hasten necrosis. Reperfusion therefore may not result in further necrosis in dogs or in humans with chronic coronary artery disease, both of whom have abundant collaterals.

Clinical Implications
There are several clinical implications of these findings. Patients with AMI could be triaged on the basis of the a priori determination of the “worst-case scenario” IS. We speculate that those with extensive collateral MBF may not need to undergo emergent revascularization as long as they are hemodynamically stable. Their infarct-related artery could be opened electively, and they could be pretreated with agents that reduce oxygen free radical production and reperfusion injury or have direct effects on microvascular flow. Meanwhile, they could be managed with drugs such as intravenous β-blockers to reduce myocardial O$_2$ consumption and thus IS.

In comparison, collateral perfusion could be maximized in patients with large RAs who are not candidates for emergent thrombolysis or angioplasty while they are being prepared for bypass surgery.

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