ORIGINAL PAPER

Gated SPECT imaging to detect changes in myocardial blood flow during progressive coronary occlusion

Timothy F. Christian · Kevin Peters · Bradly Keck · Jill Allen · Thomas Owens · Babul Borah

Abstract *Background*: The ability to track dynamic changes in myocardial blood flow (MBF) and wall motion with serial gated perfusion imaging may be a limiting factor in assessing new therapies. The purpose of this study was to determine whether gated Tc-99 m sestamibi (MIBI) SPECT imaging can track small changes in MBF in a model of progressive ischemia. *Methods*: Eight pigs (20 kg) underwent lateral thoracotomy for placement of an ameroid constrictor on the left circumflex coronary artery (LCX) and indwelling femoral and left atrial catheters for serial microsphere determinations of absolute MBF. Animals underwent concurrent left atrial microsphere and Tc-99 m sestamibi (0.3 mCi/Kg IV) injections at weekly intervals over 6 weeks per animal. Gated SPECT imaging was acquired for each injection using high resolution collimation and standard processing. The animals were sacrificed on day 42. Mean signal intensity (SI) from regions of interest (ROI) corresponding to control and ischemic MBF by microspheres was measured for three SPECT short-axis images. Mean contrast ratio (MCR) was calculated from the ratio of ischemic to control SI per slice. Regional wall motion (RWM) from gated images was scored 1–5 using a 16 segment model and a score index (RWMI) was calculated. *Results*: MBF decreased progressively (27% below resting values [$P < 0.0001$]) but with a clear and significant partial recovery by day 42 (13% improvement from peak ischemia, [$P < 0.01$]). SPECT perfusion and gated RWM closely paralleled the dynamic pattern of MBF caused by the ameroid constrictor. SPECT MCR decreased 21% from baseline scans in the LCX territory ($P < 0.0001$) and improved 11% from peak ischemia ($P < 0.01$) while the gated RWMI (1.0 at baseline) peaked at 1.36 and improved to 1.13 by day 42. *Conclusion*: Gated SPECT-a technique readily available-tracks dynamic changes in MBF closely with both perfusion and RWM. For trials of new therapies for the alleviation of chronic ischemia, these findings have direct implications for measuring efficacy.

Keywords Myocardial perfusion · Gated SPECT · Myocardial blood flow · Cardiac Imaging

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Introduction

The augmentation of myocardial blood flow (MBF) with novel approaches continues to evolve at a rapid pace. The increases achieved in MBF with these techniques may be less than those seen with percutaneous coronary interventions and bypass surgery [1] and so present quantitative challenges. Perfusion imaging is commonly employed clinically as a serial testing tool but controlled temporal reproducibility data is limited [2–4]. Investigators have extrapolated the use of clinical serial perfusion imaging to assess new anti-ischemic therapies with mixed results [5–9]. The purpose of this study was to determine whether serial imaging with gated Tc-99 m sestamibi SPECT can accurately reflect the relatively small changes in MBF in the setting of chronic myocardial ischemia as compared to myocardial blood flow determined by microsphere injection.

Methods

Model preparation

All animals received humane care and all members of the animal care team underwent animal care and use training. This study was reviewed by the Mayo Clinic Institutional Animal Care and Use Committee and complied with all institutional guidelines of the Mayo Clinic for animal research. Sus scrofa swine ($n = 10$) were anesthetized with IV xylazine and pentabarbitol and intubated for mechanical ventilation. The femoral artery was isolated and an indwelling silastic catheter introduced and externalized to the skin surface. A lateral thoracotomy was performed for surgical dissection of the left circumflex artery (LCX) for placement of an ameroid constrictor [10]. This is a ring like structure (with a gap to allow placement around a vessel) with a rigid outer surface and a soft porous inner surface. The inner material swells over time on exposure to fluid, progressively narrowing the bore of the ring and producing passive vessel constriction. A silastic catheter was placed in the left atrium and externalized for subsequent microsphere injections during the course of the experiment. The animals were allowed to recover and were housed 42 days at which time they were sacrificed. Two pigs died during surgery to give a total of eight animals who completed

the protocol. The left atrial catheter clotted off on one animal precluding a final MBF determination.

Protocol (Fig. 1)

Following the completion of surgery, pigs were injected with 1 ml of sonicated microspheres (IMT Stason Labs, Irvine, CA) for myocardial blood flow measurement at baseline. Within 10–15 min, Tc-99 m sestamibi (0.3 mCi/Kg) was injected IV and imaging commenced 10 min later. This process was repeated after day 21 and then weekly until sacrifice, for a total of five SPECT determinations per animal. Constriction of the ameroid was inferred from microsphere values in the LCX territory and subsequent myocardial necrosis by triphenlytetrazolium chloride (TTC) staining.

A. Infarct size measurement

Because myocardial necrosis impacts upon sestamibi uptake and retention [11–13], it was considered important to observe the degree of infarction that a proximal ameroid may produce. Following sacrifice, the heart was excised and sliced into 1 cm thick short-axis slices. These were stained with 1% TTC, digitally photographed and displayed on a high resolution monitor. Infarct area was planimetered using an image analysis software package (NIH Image) and expressed as a percentage of the left ventricle [14].

B. Microsphere determination of absolute MBF

Approximately 5 million fluorescent colored microspheres (1 ml) with a diameter of 15 μm (Nu Flow, IMT labs, Irvine CA) were injected into the left
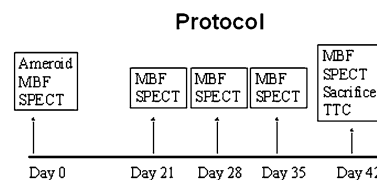

Fig. 1 Schematic representation of the protocol timeline. Five determinations of myocardial blood flow (MBF) were made concurrent with SPECT imaging over 42 days. TTC = tetrazolium chloride staining, SPECT = single photon emission computed tomography

atrium with simultaneous withdrawal of a reference sample at a rate of 7.75 ml/min × 2 min from the descending aorta with a different color for each determination of MBF. Following sacrifice, the 1 cm thick short-axis slices were sectioned radially and further divided into endocardial and epicardial zones. Segments and reference samples were sent for ultracentrifugation and analysis by an outside commercial laboratory (IMT Labs, Irvine CA). Calculation of absolute MBF was done using conventional formulae [15, 16] with the use of a reference blood sample withdrawn at a known rate during microsphere injection. The values per segment were plotted circumferentially for all five determinations and the ischemic region of the lateral wall was identified. Occlusion MBF was taken as the average of two segments for three short-axis slices (apical, mid-ventricle, and base) unless the zone was confined to one segment. Epicardial and endocardial values were averaged to obtain transmural MBF. Control zone values were taken from the unaffected inferoseptal region. Because rest control MBF can change as a result of ischemia and indwelling catheter line infection/inflammation, MBF values were normalized in all cases to the control zone at midventricle for each determination.

C. Tc-99 m Sestamibi gated SPECT

Images were acquired 10 min following injection of the tracer with a dual headed camera (El-Scint Cardial, GE, Milwaukee, WI) equipped with high resolution collimators. Each head rotated 90 degrees acquiring 15 images at 60 s per image. Acquisition was gated to R wave and acquired into 16 temporal frames which were subsequently merged into eight frames over the cardiac cycle [17]. Perfusion SPECT images were reconstructed using standard filtered background projection and displayed in vertical and horizontal long axis views and short-axis views. Attenuation correction was not available on the imaging system.

All analyses were performed off three short-axis views which were selected from landmarks previously described [18] using a quantitative image software package (Cine, GE Medical Systems, Milwaukee, WI). The mean contrast ratio (MCR) was calculated from the mean signal intensity (SI) from a

region of interest (ROI) in each SAX slice within the area of occlusion divided by the mean SI in the control zone taken at midventicle (Fig. 2). Three values were generated from the three short-axis slices per animal for each time point.

Regional wall motion analysis

A 16 segment model based was scored subjectively by a single blinded observer for both motion and brightening (as a surrogate for thickening). To be scored normal [1] a segment had to show both motion and brightening. Motion without brightening was scored 2–5 depending on the degree on motion abnormality (2 = normal or mild hypokinesis, 3 = moderate, 4 = severe, 5 = akinesis or dyskinesis). A global score (RWMI) was calculated per study by summing the scores of each segment and dividing by the total.

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Analyses were performed for comparison of measures over five time periods using one-way analysis of variance. Post-hoc comparisons of paired
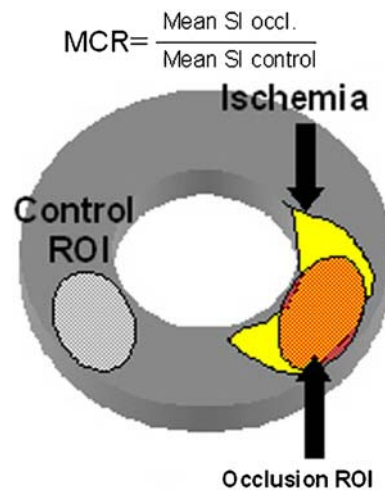

Fig. 2 Method of determining the mean contrast ratio (MCR) from SPECT short-axis images. Mean SI from the ischemic zone is contrasted to mean SI from a control zone to form the ratio of the two zones. Three slices (apex, mid-ventricle and base) were analyzed per animal. The same procedure and locations were used for the microsphere measures to normalize MBF SI = signal intensity, ROI = region of interest, Occl = coronary occlusion zone

Table 1 Pathological and imaging variables by animal[a]

Animal	Infarct size (%LV)	Baseline MBF	Peak ischemic MBF	Final MBF	Baseline SPECT MCR	Peak ischemic MCR	Final MCR	Peak ischemic RWMI	Final RWMI
1	11.6	1.12	0.72	0.87	1.09	0.60	0.76	2.3	1.5
2	11.3	0.91	0.58	0.79	0.89	0.73	0.80	1.8	1.4
3	1.3	0.81	0.55	0.86	1.08	0.73	0.89	1.2	1.0
4	0	0.99	0.83	0.84	1.09	0.81	1.01	1.1	1.0
5	1.6	0.98	0.75	–	0.90	0.73	0.84	1.1	1.0
6	2.6	1.05	0.86	0.95	0.99	0.88	0.96	1.1	1.0
7	9.5	0.96	0.59	0.79	1.02	0.61	0.78	1.3	1.0
8	1.9	1.00	0.79	0.94	0.92	0.79	0.88	1.3	1.1
Mean	5.0 ± 4.9	0.98 ± 0.09	0.71 ± 0.12	0.86 ± 0.06	1.00 ± 0.09	0.74 ± 0.10	0.87 ± 0.09	1.4 ± 0.4	1.1 ± 0.2

[a] Baseline average values: normalized MBF = 0.99 ± 0.23, MCR = 0.97 ± 0.11, RWMI = 1.0 ± 0

Each MBF and MCR value reflects the average of three measures: apical, mid-ventricular and basal

time points were also performed using a Fischer's LSD test. A two-tailed unpaired t-test was used to compare SPECT MCR values to microsphere-derived measures of MBF. A P value <0.05 was considered significant. Intraobserver reproducibility of the SPECT MCR measure was assessed by paired measures in four animals. MCR values were measured in apical, mid-ventricular and basal slice locations for three points in time: baseline, day 28, and day 42 for a total of 36 paired measures.

Results

A total of eight pigs were successfully completed. Initial animal weight was 21.7 ± 1.7 Kg and weight at sacrifice was 34.7 ± 6.8 Kg. There was a trend for heart rate (HR) to decrease over the course of the protocol but this was not statistically significant (HR baseline = 99 ± 21 bpm, HR sacrifice = 84 ± 16 bpm). Infarct size was 5.0 ± 4.9%LV with a range of 0–11.6%LV (Table 1) and usually demonstrated a mosaic pattern in that patches of necrosis were intermixed with viable tissue. There was no evidence of transmural infarction in any animal. One animal had no evidence of necrosis.

Myocardial blood flow results

The average myocardial segment mass for calculation of MBF from microspheres was 3.2 g (24 segments

per pig). Absolute MBF at baseline was 0.73 ± 0.52 ml/min/g (endocardial MBF = 0.78 ± 0.53 ml/min/g, epicardial = 0.69 ± 0.51 ml/min/g). Control zone MBF increased significantly after initial surgery (week 3 mean MBF = 1.34 ml/min/g, final MBF = 1.48 ml/min/g). The ratio of occlusion MBF/ Control MBF (normalized MBF) is shown in Fig. 3 and Table 1. Overall, MBF varied significantly from baseline through the protocol (ANOVA $P < 0.0001$). Peak ischemia was reached between weeks 4 and 5 and was significantly lower (27% decrease) than baseline MBF (0.99 ± 0.23–0.73 ± 0.15, $P < 0.0001$). There was a significant recovery of 13% in MBF by week 6 (0.73 ± 0.15–0.86 ± 0.11, $P < 0.01$).

SPECT perfusion

Technically satisfactory perfusion images and gated images were obtained on the eight surviving pigs approximately 20 min following completion of the microsphere MBF determinations. There was no significant change in HR between the two modalities. The intraobserver variability of the SPECT MCR measurement was 0.029 and the 95% confidence interval for a significant change for this measure was 0.048. There was no significant difference overall between MBF by microspheres and SPECT MCR (MBF = 0.85 ± 0.21, MCR = 0.85 ± 0.13, P = NS). This was true for all five time points and for slice location (Fig. 4). The MCR closely paralleled the changes in MBF over the five measurements (Fig. 3
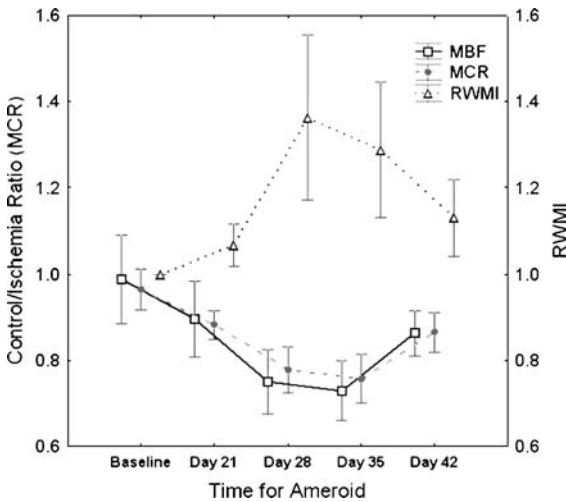
Fig. 3 Summary results of the the two imaging parameters and the microsphere-derived normalized myocardial blood flow (MBF) values over five points in time. Data points represent mean values and whiskers represent 95% confidence intervals of the mean. For all three measures, the overall values changed significantly over time ($P < 0.0001$ for all three). Significant differences by post-hoc analysis exist for all 3 measures at weeks 3–4 compared to baseline ($P < 0.0001$) and week 6 compared to weeks 3–4. ($P < 0.01$). MCR = SPECT mean contrast ratio, RWMI = regional wall motion index by gated SPECT imaging

and Table 1) with a significant decrease at peak ischemia of 21% compared to baseline (0.97 ± 0.11–0.76 ± 0.13, $P < 0.001$). Following peak ischemia there was a significant increase in the MCR value of 11% (0.76 ± 0.13–0.87 ± 0.11, $P = 0.0017$) which
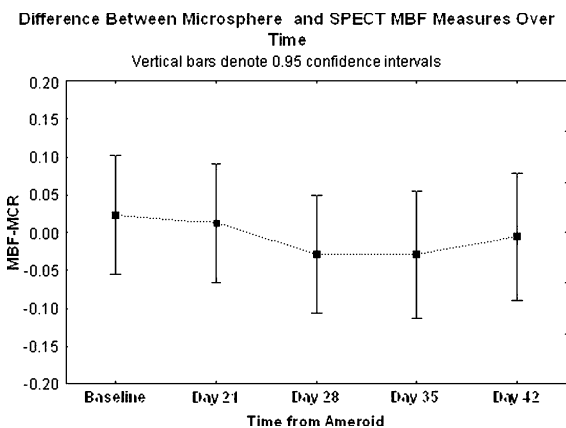

Fig. 4 Mean values (squares) and 95% confidence intervals (whiskers) for the difference between myocardial blood flow (MBF) by microspheres and SPECT myocardial contrast ratio (MCR) measures. Overall, there was no significant difference between these values overall, or by specific time points

paralleled the increase in MBF. All eight animals had an increase in the SPECT MCR from the ischemic nadir to the final acquisition that was greater than the reproducibility of the MCR measurement (Table 1).

Gated SPECT regional wall motion

The wall motion score (where 1 is normal wall motion and 5 is akinetic/dyskinetic) changed significantly over the 6 weeks following placement of the ameroid constrictor on the left circumflex artery (Fig. 3) with a significant increase in RWMSI (1.0 ± 0–1.36 ± 0.45, $P < 0.0001$ by ANOVA) by day 28 and significant recovery by day 42 (1.36 ± 0.45–1.13 ± 0.21 $P < 0.01$). This inversely paralleled the findings for MBF over the same period. The presence of necrosis did not preclude regional wall motion (RWM) recovery. Pigs with larger infarcts (9–11% LV) had the greatest recovery in RWM (Table 1). However, infarct size was small in all animals.

Discussion

Imaging of myocardial blood flow has evolved from a diagnostic tool for the detection of epicardial vessel obstruction to an efficacy end-point for trials examining novel approaches for revascularization. These therapies and their highly localized delivery place extraordinary demands on imaging techniques which were not designed to provide precise measurements of myocardial blood flow in small segments of myocardial tissue. The performance of low resolution conventional imaging methods in clinical trials for the measurement of induced changes in MBF by these agents has been mixed [5–9, 19]. The improvements in blood flow which occur following the administration of growth factors is generally believed to be generated in vessels less than 200 μm in diameter [20]. Evaluating efficacy is therefore a challenge for cardiac imaging and particularly for SPECT where resolution is 1–1.5 cm.

Visual estimates of isotope uptake have been used for quantifying the small changes in MBF from growth factors administered to patients [5–7, 19]. Isotopes cannot quantify MBF in absolute terms due to photon scatter, attenuation and low spatial

resolution but there are semi-quantitative methods can be applied as in the present study.

This study demonstrates that SPECT perfusion imaging in combination with gated wall motion provides a fairly clear serial representation of dynamic changes in MBF as might be seen in patients with ischemic heart disease. The MCR from the perfusion images, which provides a measure of mean SI in an ROI compared to a control zone, tracked mean changes in MBF closely in both progressive ischemia and presumed collateral flow development.

The changes in MBF in this model were relatively modest with a maximum reduction at weeks 4–5 of 27% from baseline and a modest recovery to climb within 13% of baseline. These changes did exceed the reproducibility of the measure, however. Despite these modest changes, the changes in wall motion by gated SPECT were significant. The changes in RWMI have also been found by other researchers to be a strong indicator of both the damage and recovery phases in the ameroid-induced pig model of ischemia. Zhang et al. [21] found a very similar relationship between RWM determined by gated SPECT and the recovery of ischemic area and ejection fraction. Lopez et al. [22], using the ameroid-induced ischemic pig with open chest echocardiography, found a good relationship existed between wall motion and MBF during the recovery phase, with the addition of fibroblast growth factor. Sato et al. [23], also in the ameroid-induced pig, found that wall motion and wall thickening were indicators for the recovery phase of the model.

Other techniques can provide both perfusion and regional wall motion data regarding therapies targeted to alter myocardial blood flow. Echocardiography and magnetic resonance imaging have demonstrated significant improvements in wall motion score in chronic ischemic animal models in both control and target treated regions [24–26]. Contrast Echocardiography has demonstrated measurable improvements in chronically ischemic myocardium which have correlated reasonably well with microsphere determinations of flow out to 48 days [25]. These studies were confined to two imaging time points and did not track dynamic changes, as in the present study.

While subjective scoring systems for perfusion changes may be successful, some degree of quantitation is likely necessary given the overall small changes in MBF reflected in this model and in human

trials [9, 27, 28]. Rosengaart et al. found no change in perfusion in patients receiving vascular endothelial growth factor by SPECT as measured by a well validated subjective method employed for ischemic heart disease [19]. However, gated wall motion improved significantly suggesting the changes in perfusion are likely too subtle to be accurately reflected in purely subjective segmental analysis. The combination of semi-quantified SPECT perfusion with an index of subjective RWM may provide an effective combination to assess small degrees of MBF change.

The changes in SPECT perfusion were measurable despite the relatively low resolution of the technique in the setting of minimal infarction. Large chronic infarcts will not perform as well due to the dependence of viability for Tc-99 m sestamibi uptake [14]. Recently, serial gated SPECT imaging has been performed to evaluate the success of various stem-cell strategies in patients and animal models of infarction with mixed results [29–31]. This type of therapy is based on a different physiologic principle: the change in viability status through altering the cellular content of the infarct. The principles for the noninvasive quantitation for this change in viability [4] are quite different than the method used in the present study to track changes in MBF in a viable but ischemic zone. Altering viability may be the standard of care of the future and imaging methods designed to specifically target viability may provide effective measures. The animal model used in this study was based upon therapies that change blood flow and is not directly applicable to cellular therapies.

Limitations

No attenuation correction algorithms were used in the imaging protocol, which may assume more importance for clinical use where body habitus is more variable. Serial imaging of the same subject with the same tracer dose should produce a constant attenuation pattern. The infarcts generated in the present study were small. Performance of serial gated-SPECT for RWMI may not perform as well in the setting of large perfusion defects [32]. Anatomic analysis for collateral development was not performed to confirm the etiology for the increase in MBF from peak ischemia to sacrifice. However, the

development of collateral circulation in this ameroid swine model has been demonstrated previously [27, 28] and the increase in MBF following an ischemic nadir in this study was likely to be the consequence of collateral development. While it is speculated that these results can be extrapolated to animal models and human trials where changes in regional myocardial blood flow can be invoked, there was no such therapeutic modality in this study.

Radionuclide perfusion SPECT imaging is a relative technique where the SI is scaled to the peak activity in the myocardium. Serial imaging assumes the peak region to which all activity is scaled does not change from one exam to the next. This may not hold for nonselective application of a therapeutic agent and needs further investigation. However, in the present study, SPECT was able to track fairly small changes in MBF in an untreated ameroid constrictor model of chronic ischemia. This is an encouraging result regarding both the clinical and experimental use of serial SPECT perfusion imaging.

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