Vasodilator Response Assessment in Porcine Myocardium With Magnetic Resonance Relaxometry

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Background—This research describes an early preclinical study of the biophysical mechanisms governing changes in myocardial T2 during vasodilation in normal myocardium.

Methods and Results—Theoretical modeling and experimental studies in an instrumented pig model (n=7) provided measures of changes in myocardial T2, relative blood volume (BV), and microcirculation oxygen levels (%O2) during intracoronary adenosine infusion. Intracoronary adenosine increases perfusion without increasing blood volume or cardiac metabolic rate; thus, T2 elevations should reflect elevated microcirculation oxygen levels. Robust strategies were used for magnetic resonance imaging (MRI) data collection. Measures of myocardial and vascular T1 before and after Clariscan (Amersham Health) injection provided blood volume assessment. Changes in microcirculation oxygen levels were estimated via direct blood sampling from the left anterior descending (LAD) coronary vein. Perfusion changes were monitored using a Doppler flow wire within the left main coronary artery. Myocardial T2 elevations (ΔT2=17±8%) within the LAD arterial perfusion bed were related to elevations in perfusion (coronary velocity reserve=3.2±0.4) and coronary venous %O2 [Δ(LAD CV%O2) =56±11%), whereas blood volume (ΔBV=0 ±2%) and cardiac metabolic rate (Δ(heart rate x blood pressure)) =−4±11%) remained constant.

Conclusions—Myocardial T2 elevation during intracoronary adenosine infusion was significant and repeatable, caused by increases in microcirculation oxygen levels. Changes in microcirculation oxygen levels of approximately 40%O2 should be detectable by this technique. This sensitivity should suffice for differentiating normal from abnormal myocardium via measurement of myocardial perfusion reserve. (Circulation. 2002;106:2714-2719.)

Key Words: magnetic resonance imaging ■ myocardium ■ adenosine ■ oxygen ■ blood volume

Noninvasive tests to differentiate between normal and abnormal myocardium are useful to predict the clinical benefit from percutaneous or surgical revascularization in patients with coronary artery disease. Stunned myocardium is characterized by near-normal basal state perfusion and oxygen consumption.1 Basal state perfusion and perfusion reserve, however, are reduced in areas of hibernating myocardium.2,3 Currently, perfusion reserve imaging requires direct measurements of perfusion in the basal and vasodilatory state. With magnetic resonance imaging (MRI), these measurements are generally performed during the first-pass of contrast agents. The data analysis is often simplified, however, so that only indices of relative perfusion reserve are obtained.4 We hypothesize that changes in microcirculation oxygen levels during vasodilation can be used to quantitate myocardial perfusion reserve without direct measurement of perfusion.5

There are few current methods available for evaluation of oxygen levels in myocardial microcirculation, and most are invasive. Microcirculation and cellular oxygen levels (%O2) may be measured by optical reflectance spectroscopy.6 Cellular %O2 may also be assessed using proton magnetic resonance spectroscopy.7 Other imaging modalities, such as single photon and positron emission techniques (SPECT, PET), allow examination of a variety of aspects of myocardial metabolism but not %O2 directly.

Magnetic resonance allows noninvasive measurement of vascular %O2 via measurements of T2, the time constant of MR signal relaxation, within peripheral vessels8 and the coronary sinus of human volunteers.9 Elevations in myocardial T2 with vasodilation have been demonstrated in swine8 and human volunteers.10 However, this signal behavior can reflect changes in either blood volume (BV) or %O2 at the microvascular level. In this study, we characterize each contribution in a study of vasodilation with intracoronary adenosine in normal porcine myocardium.

Methods
Studies were conducted in Yorkshire pigs (Riemans Fur Ranch, St Agatha, Ontario, Canada) of both sexes by use of procedures and protocols approved by the Research Ethics Board of Sunnybrook and Women’s College Health Sciences Center.
Experimental Preparation
Each animal was premedicated using a ketamine/atropine cocktail (35 mg/kg ketamine hydrochloride and 0.05 mg/kg atropine) and masked at 5% halothane in oxygen for anesthesia induction. After endotracheal intubation, halothane was reduced to 1% to 2% and maintained at that level for the duration of experimentation. The left carotid artery was exposed and 2 sheaths were inserted. Through 1 sheath, a catheter was introduced into the left main coronary artery under fluoroscopic guidance. This catheter was used to guide both the infusion of adenosine and the placement of a flow wire into the left main coronary artery. The other sheath was used for vascular blood sampling and for insertion of a pressure catheter into the carotid artery. Heparin (2000 IU) was injected at 2-hour intervals during the procedure.

Study Procedures
A total of 7 animals were examined (40 to 45 kg) within a 3-component study. T1 and blood volume responses to adenosine were assessed using MRI. Physiological instrumentation in a closed-chest animal allowed assessment of coronary velocity reserve and changes in cardiac metabolic rate. After thoracotomy, the left anterior descending (LAD) coronary vein was cannulated for monitoring of changes in myocardial oxygenation. MR imaging was performed with only limited physiological instrumentation because available probes introduced substantial noise within the MRI suite. As these physiological measures required moving the animal, with the associated potential for shifts in instrument positioning, it was decided to use the x-ray imaging capabilities in the surgical suite to confirm their location during measurements.

The model for blood volume measurement is summarized in the Appendix. The requisite measures include myocardial and intravascular T1 within native tissue and after injection of an intravascular contrast agent.11 To reduce perfusion bias, the blood volume within each animal was estimated at multiple contrast agent concentrations after a single 2.5 cc bolus injection of Clariscan (Amersham Health).

Adenosine infusion was driven using an IMED 980 infusion pump. During MRI scanning, the pump was placed within a Faraday cage to reduce radiofrequency (RF) interference associated with the pump motor. Adenosine (Fujisawa Healthcare) was mixed at a concentration of 2 mg/mL in 0.7% saline and was infused at a rate of 2 mL/min. The corresponding infusion rate for a 40 kg animal is 100 µg · kg⁻¹ · min⁻¹, matching the dosage deemed optimal given intracoronary delivery.12 A 1-minute delay was introduced after initiation of intracoronary adenosine infusion to allow for development of the vasodilatory response.

MRI Scanning
All studies were performed on a 1.5 Tesla GE CV/i system (peak gradient amplitude of 40 mT/m, peak slew rates of 150 T · m⁻¹ · s⁻¹). The pig was oriented in a supine position within a plastic casing. A 5-inch surface coil positioned anteriorly to the heart provided signal reception. The pig forelimbs were immobilized to minimize respiratory motion effects on surface coil positioning. A plethysmograph attached to the pig’s tail provided a waveform for cardiac gating.

All images were acquired in a single short-axis slice located at the level of the mid-left ventricle. A field map was acquired to verify system tuning. Native tissue data sets were first acquired, including basal and vasodilatory state T1 and T2 data sets. Bolus injection of Clariscan (2.5 ccs) was followed by a 2-minute wait for agent distribution. Basal and vasodilatory state T1 acquisitions were then repeated. These T1 acquisitions were repeated until a total MRI scan time of 2 hours was reached (up to 4 twenty-minute delays for each animal).

Intravascular T1 (T1.iv) was estimated at the time of each T1 acquisition via withdrawal of a blood sample through the left carotid arterial cannula. T1.iv values were determined using in vitro scanning within 24 hours of withdrawal. Samples were placed within a MnCl2-doped water bath warmed to 37°C before scanning to limit susceptibility effects without adding signal. The head coil and a consistent set of data acquisition parameters were used.

MRI Pulse Sequences
The method for myocardial T1 measurement is a modification to a previously validated spiral imaging strategy for coronary sinus oximetry in humans.13 Images were acquired at 2 relatively early data acquisition times (11 and 55 ms) to increase per voxel signal-to-noise ratio. The spiral imaging parameters were selected to reduce blurring of chamber blood signal across the endocardial border.

The T1 measurement uses a spiral-imaging version of the Look-Locker sequence, which has been validated in skeletal muscle of human volunteers.13 It consists of a nonselective inversion followed by a train of eight 15° excitations. The delay between excitations was 120 ms. The total imaging time is about 80 seconds per data set. The signals acquired at each excitation time point were used to construct separate images. A RF cycling scheme was applied to subtract the steady-state term in the T1 recovery. The signal variation across different acquisition delays can then be modeled as a signal decay. No compensation for RF sampling effects on the measured relaxation was applied because the correction factor cancels out of the BV measurement.14

All images were acquired using 6 slew-rate–optimized spiral k-space trajectories over a 24 cm field of view (4096 data samples per 16.4 ms spiral interleaf). Voxel dimensions were 1.5 mm × 1.5 mm × 4.8 mm. A systolic cardiac phase was chosen for data acquisition because the thicker myocardial wall reduces partial voluming. Six data averages were acquired to reduce respiratory motion artifacts. The absence of significant chest wall image artifacts in localization and T1-weighted images justified the use of this strategy over more involved motion compensation techniques. Three RR intervals were allowed for signal recovery between data acquisitions (roughly 2 seconds for a 95 bpm heart rate).

Hemodynamic Measurements
The pig was transported to the surgical suite within 45 minutes of the end of MRI scanning. The location of the intracoronary catheter within the left main coronary artery was verified and/or re-positioned at this time using x-ray fluoroscopy for guidance. Animal preparation included insertion of a Doppler flow wire (Cardiometrics) into the left main coronary artery for coronary velocity reserve (CVR) assessment. A 6-F Miller catheter placed into the left carotid artery provided blood pressure monitoring, with waveform display on a calibrated LeCroy digital oscilloscope. Heart rate was monitored using a pulse oximeter.

After animal preparation, the vasodilator response assessment was completed in 2 stages. First, CVR and heart rate and blood pressure responses to intracoronary adenosine were assessed with the chest closed to verify the perfusion response and to examine coupling between CVR and cardiac metabolic rate. Next, the LAD coronary vein was cannulated using a 22-gauge angiocatheter after left lateral thoracotomy and pericardial excision. Blood samples were withdrawn under basal conditions and during adenosine infusion to assess the myocardial oxygenation response. The sample oxygenations were determined optically.

MRI Data Analysis
Image analysis used a single region of interest (ROI) placed manually within the perfusion bed of the LAD coronary artery. The ROI volumes were on the order of 0.3 ccs (roughly 28 independent voxels given 1.5 mm × 1.5 mm × 4.8 mm resolution). To reduce blurring from intracardiac blood signal and physiological noise in BV and T1 relaxation estimation, the ROI covered only the middle 50% of the myocardial wall. This ROI placement may also reduce susceptibility differences in BV and %02, should be most apparent in the subendocardium.15–17

A ROI for T1 analysis was copied across the echo time pair because the cardiac and respiratory phases were consistent across the data set. A ROI for T1 analysis was repositioned for each image within the data set because the images were acquired at different cardiac phases. The relaxation times were estimated from plots of logarithms of the signal intensities versus the data acquisition times.
using a linear least squares-fitting algorithm. No data point in which per voxel signal-to-noise ratio was less than 10 was included into the data fitting. The quoted uncertainties in the relaxation times reflect noise propagation through the data fitting.

Measurement of Blood Volume

Myocardial and vascular T1 pairs were propagated through the equation shown in the Appendix for blood volume estimation. A linear fit was applied to the BV and T1 data pairs from all animals to extract the slope, which is proportional to perfusion, and the y-intercept, which reflects the true blood volume.11

Statistical Methods

The significance of T2 changes was assessed via paired 2-tailed Student’s t test and on a per-animal basis, assuming known standard deviation of T2 (σT2). σT2 is about 5% from on-going human studies. The significance of changes in linear fit parameters for BV measurement was estimated assuming normal distribution after calculation of the weighted mean parameter change and standard deviation across all animals.17a Significance in hemodynamic parameters was assessed via t test.

Results

MRI Scanning

T2 measurements are summarized in Table 1. On the basis of t test, T2 changes during adenosine were statistically significant (P=0.002). On a per-animal basis, T2 changes were borderline significant in 2 animals (P=0.09) and highly significant in the remaining animals (P<0.005). Typical T2 images are shown in Figure 1. T2 increased from 42±0.4 ms to 53±0.4 ms, a percentage increase of 26%.

BV measurement was not technically successful within 1 animal. A total of 18 BV measurements at different vascular T1 were obtained from the remaining 6 animals (Figure 2). The weighted mean y-intercept did not change during vasodilation (weighted mean difference=0±2%), reflecting a constant tissue blood volume. The weighted mean slope of the BV versus T1 relation increased significantly during vasodilation (weighted mean difference=0.010±0.003; P<0.02), reflecting an increase in perfusion. Images and data fittings from a typical blood volume measurement are displayed in Figure 3.

Hemodynamic Measurements

One animal died en route to the surgical suite. Blood pressure measurements were technically successful in 5 of 6 animals. Loss of animal stability after venous cannulation limited vasodilatory state blood sampling to 3 animals only.

All measurements are summarized in Table 2. Perfusion increased significantly during vasodilation (P=0.001), associated with a mean coronary velocity reserve of 3.0±0.4. Reductions in cardiac metabolic rate were not statistically significant (P=0.132). Considerable increases in coronary venous %O2 were characteristic of vasodilation (P=0.013).

Discussion

This is the first experimental study within an in vivo model relating both myocardial T1 and T2 changes during vasodilation to underlying mechanisms, namely changes in %O2 and BV. Prior studies in vivo aimed to characterize decay elevations, with less emphasis on the underlying biophysical characteristics.10,18,19 Myocardial R2 (R2 = 1/T2) changes during intracoronary adenosine have been correlated with myocardial perfusion reserve (MPR) within instrumented pigs.9

The underlying changes in tissue %O2 and blood volume were not ascertained. The T2 change with MPR in that study (∆T2 = −0.94 s−1 × MPR) predicts a T2 elevation of 16% when basal T2=50 ms and MPR=3, in accordance with our results.

The observed parameter responses to intracoronary adenosine infusion are in accordance with the literature. The presence of a perfusion response with only minor reductions...
in cardiac metabolic rate ($\Delta$HR and $\Delta$BP about $-5\%$ at an infusion rate of 100 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) has been established clinically. The extent of the perfusion response is suggestive of near-maximal adenosine vasodilation. That tissue blood volume remains constant has been noted in studies using radio-labeled red blood cells and plasma markers and ultrasound microbubbles. Elevated coronary venous oxygenation from 25% to 90% has been observed via direct venous blood sampling within pigs. This result is consistent with our own volunteer studies of intravenous dipyridamole effects.

Our results demonstrate that intracoronary (IC) adenosine increases coronary venous $\%O_2$, whereas BV remains constant. It is well known that increases in vascular $\%O_2$ elevate vascular $T_2$. That increases in microcirculation oxygen levels elevate myocardial $T_2$ has been demonstrated in vitro. We conclude that elevations in myocardial $T_2$ during IC adenosine reflect elevations in microcirculation oxygen levels, and thus perfusion reserve.

**Predicted $T_2$ Elevation During Adenosine Infusion**

A model of the myocardial MR signal relaxation relates myocardial $T_2$ changes to changes in volume and $T_2$ in the intravascular and extravascular compartments. Basal and vasodilatory state signal amplitudes at acquisition times of 11 and 55 ms may then be simulated and passed through the data fitting process.

The model depends on a series of physical parameters. For basal and vasodilatory state intravascular volumes, we consider a range of blood volumes (5% to 12%), consistent with the literature. Intravascular $T_2$ may be estimated from oximetry measurements within larger vessels; a basal state oxygen level of 27% $O_2$ maps to a vascular $T_2$ about 39 ms and a vasodilatory state oxygen level of 82% $O_2$ maps to a vascular $T_2$ about 183 ms. Parameters of the extravascular space include a relative volume of 1/3 intravascular volume and $T_2$ of 40 ms. A water exchange rate of 7 per second is assumed.

The predicted $T_2$ responses to adenosine are summarized in Table 3. These predictions overlap the experimental measurements of $T_2$ changes (Table 1). The absolute $T_2$ estimates are similar to those observed experimentally.

**Oximetric Assessment of Perfusion Reserve**

A challenge in perfusion imaging is determining whether perfusion deficits during vasodilation originate from viable yet damaged myocardium or from infarction. Sensitivity to 2-fold reductions in MPR is considered necessary. A recent study demonstrated that this level of sensitivity was possible with the use of MRI. It is the purpose of this section to compare the sensitivities of $T_2$ and perfusion imaging for perfusion reserve assessment.

We estimate sensitivity of myocardial $T_2$ to perfusion reserve using the myocardial relaxation model and an oximetry.
metric expression for perfusion reserve derived from Fick’s law. 5 With $\sigma T_2$ of 5% from on-going human studies, $T_2$ changes greater than 10% are required for confident detection. At an intravascular volume about 8%, a 10% elevation in myocardial $T_2$ maps to an elevation in microcirculation $\%O_2$ level about 40% $O_2$. The relation between MPR and $\%O_2$ is displayed in Figure 4. A $\%O_2$ value about 40% $O_2$ maps to a 3-fold reduction in MPR.

On the basis of this analysis, one would expect MRI perfusion imaging to provide a more sensitive evaluation of perfusion reserve than that available currently using myocardial $T_2$. A 3-fold reduction in MPR, however, should allow differentiation between normal and hibernating myocardium. With further technical development, sensitivity to MPR may be increased, and the additional metabolic information contained within $T_2$ relaxation may prove to hold value.

Study Limitations

Three main limitations with this study have been identified. First, there was some inconsistency between heart rate and blood pressure responses during adenosine vasodilation (see pig 5, Table 2). Shifts in intracoronary catheter positions during animal transportation could affect adenosine delivery to cardiac pacing cells. Also, more thorough sampling of the basal and vasodilatory state blood volume versus vascular $T_2$ relations would improve data fitting on a per animal basis. This approach is difficult to implement, given a Clariscan half-life about 50 minutes. Finally, more invasive approaches could improve $O_2$ and volume assessment. 6, 20 The extent of invasiveness, however, may prevent measurements within multiple physiological states.

Conclusions

Theoretical modeling and experimental studies in an instrumented pig model provided interpretation of adenosine effects on MR relaxation times in the myocardium. Elevations in myocardial $T_2$ during intracoronary adenosine infusion are significant and repeatable. $T_2$ elevation is caused by increases in microcirculation oxygen levels, with tissue blood volumes remaining constant, as determined from $T_1$ measurements. Changes in microcirculation oxygen levels about 40% $O_2$ should be detectable. This sensitivity should suffice for differentiating normal from abnormal myocardium via non-invasive oximetric estimation of myocardial perfusion reserve.

Appendix

Blood volume measurement using $T_1$

The model for blood volume measurement assumes rapid water exchange between intravascular and extravascular spaces. 6, 20 The condition of fast water exchange seems to be met when $T_1$ is greater than 150 ms. 31 Model application requires measurements of myocardial $T_1$ ($T_{1,myo}$) and intravascular $T_1$ ($T_{1,iv}$) within native (nat) tissue and post-injection (pc) of an intravascular contrast agent.

$$BV (\%) = \frac{1}{T_{1,myo,pc}} - \frac{1}{T_{1,myo,nat}}$$

Perfusion during the time course of data sampling introduces bias into the volume estimate. The effect is manifest as a linear dependence of the measured BV on intravascular $T_1$. 6, 20 The slope is proportional to perfusion. The y-intercept (B) provides a perfusion-sensitive estimation of volume. The change in BV during vasodilation is reflected within the percentage change of vasodilatory to basal state B values [(B$_{vaso}$ − B$_{baso}$)/B$_{baso}$].

Perfusion-insensitive volume estimation may also be accomplished through application of a slice-selective, rather than a non-selective, inversion pulse. 26 The need for synchronization of the slice profiles of the inversion pulse and each small-tip angle excitation could inhibit application in vivo. As a result, we favor the estimation of B from multiple measurements at various $T_{1,myo}$ values.

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References


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