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Myocardial washout of sonicated iopamidol does not reflect the transmural distribution of coronary blood flow

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It has been shown in previous studies that myocardial contrast echocardiography provides quantitative information on coronary blood flow. However, the ability of contrast echo to assess the transmural (endo/epicardial) distribution of blood flow is still debated. To test this hypothesis, the left circumflex coronary arteries of six anaesthetized open-chested dogs were cannulated and perfused with blood from the femoral artery. At different rates of coronary blood flow, during adenosine-induced coronary vasodilation, sonicated iopamidol and radionuclide labelled microspheres were injected into the coronary cannula, immediately proximal to a mixing chamber. Two-D echo images were digitized and myocardial time–intensity curves were obtained for the endocardial, mid- and epicardial layers. A good correlation existed between contrast washout of the entire ventricular wall and coronary flow ($r=0{\cdot}85$). However, the washout rate from the endo-, mid- and epicardial layers showed weak correlations with corresponding regional blood flows measured by microspheres ($r=0{\cdot}56, 0{\cdot}71$ and $0{\cdot}58$, respectively). No significant relationship was found between the endo/epicardial washout ratio and the corresponding flow ratio by microspheres. Thus, measurement of the transmural distribution of coronary blood flow by myocardial contrast echocardiography remains an elusive goal.

Introduction

Previous experimental and clinical studies have shown that it is possible to derive quantitiative information on coronary blood flow by myocardial contrast echocardiography. In particular, the rate at which the echographic contrast effect disappears from the myocardium, usually referred to as myocardial contrast washout, has been shown to be prolonged in the presence of severe coronary stenoses[1–3]. Furthermore, a good correlation was found between the transit time of myocardial contrast and coronary flow rate, when measured independently by electromagnetic flowmeters and radionuclide labelled microspheres[4]. More recently, the relationship between myocardial echo contrast washout and coronary blood flow was studied in a canine model in our laboratory. Again, a good correlation was found between the rate of myocardial contrast washout and actual coronary blood flow[5].

In the light of these results — and considering the high spatial resolution of two-dimensional echocardiography — it is tempting to test the hypothesis that myocardial contrast echocardiography may allow quantification of coronary blood flow in the different myocardial layers. Studies dealing with this problem have so far been controversial. Specifically, the transmural (endo/epicardial) distribution of the echo contrast effect was studied by Cheirif *et al.* in a canine model with coronary vasodilation. A relative underperfusion of the inner subendocardial layers was observed by contrast echocardiography in the presence of coronary stenoses[6]. Similarly, a subendocardial underperfusion was observed by Lim *et al.* in patients with coronary artery disease during myocardial ischaemia induced by rapid atrial pacing[7]. Conversely, no significant changes in the transmural distribution of several contrast echo parameters were observed by Kaul *et al.* in the presence of severe subendocardial underperfusion in dogs[8]. Thus, the purpose of this study was to investigate whether myocardial contrast echocardiography allows quantification of transmural distribution of coronary blood flow.

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Methods

ANIMAL PREPARATION

The animal preparation has been previously described[5]. Six mongrel dogs of both sexes (19–25 kg) were initially sedated with morphine sulphate (2·5 mg . kg^{-1} s.c) and 1 h later anaesthetized with alpha-chloralose (100 mg . kg^{-1} i.v.). The dogs were ventilated with oxygen-enriched room air by a positive pressure ventilator (Harvard respirator, Model 613). A pressure-tip catheter (Millar 7 F) was inserted into the abdominal aorta through the right femoral artery. In order to avoid the increased heart rate associated with open chest preparation, A-V heart block was produced by local formaldehyde injection[9], and the heart was paced via a wire positioned into the right ventricular apex. A left thoracotomy was performed, the pericardium excised and the heart suspended in a pericardial cradle. The left circumflex coronary artery was dissected free from the surrounding tissues and a double-lumen steel cannula was inserted into the vessel; heparin sodium (1000 U . kg^{-1} i.v.) was administered

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before coronary cannulation. The circumflex coronary artery was perfused with blood from the left femoral artery by a calibrated roller pump through one lumen of the cannula and maintained at body temperature by a thermostatic system. Circumflex coronary flow was monitored by an electromagnetic flow transducer (Zepeda SWF-4RD) and coronary arterial pressure at the tip of the cannula was measured through the second lumen of the cannula via a strain gauge manometer. A mixing chamber was positioned immediately proximal to the coronary cannula. After coronary cannulation, adenosine was continuously infused into the circuit by a perfusion pump, at doses ranging from 0·6–1·8 mg . min^{-1}. To test the efficacy of the adenosine dose in abolishing coronary autoregulation, coronary perfusion was stopped for 20 s: the lack of hyperaemic response when perfusion was restored was considered to reflect maximal vasodilation. If moderate coronary hyperaemia occurred, the dose of adenosine was increased and the procedure repeated. A partial right thoracotomy was also performed to optimize the echocardiographic examination. Before and during the injection of the contrast agent, haemodynamic parameters and two ECG leads were recorded on paper.

ECHOCARDIOGRAPHIC EXAMINATION

Two-dimensional echocardiograms were obtained using a commercial sector scanner (77020, Hewlett Packard), operating at 5 MHz. The transducer was positioned on the right ventricle via the right thoracotomy and was oriented so as to obtain a short axis view of the left ventricle. Gain setting controls were adjusted to optimize image quality at the onset of the first experiment, and were maintained constant throughout all the studies. Echocardiographic images were recorded on a 0·5 inch VHS videotape for subsequent of line analysis.

ECHOGRAPHIC CONTRAST AGENT

Sonicated iopamidol (Iopamiro 370, Bracco) was used as the echocardiographic contrast agent in all experiments, and sonication was performed according to a well established protocol[10,11]. Briefly, the tip of the sonicator horn (Heat System W 220, U.S.A.) was introduced (3 mm depth) into 8 ml of contrast medium, contained in a 10 ml plastic syringe. Three periods of sonication, lasting 10 s each, were performed in rapid succession. The microbubbles produced by this procedure are reported to have a mean diameter of $5·5 \pm 3·0$ μm[11]. One millilitre of contrast agent was hand-injected as a bolus immediately upstream of the mixing chamber. If the myocardial echo contrast effect was too weak to examine visually, the dose of contrast agent was doubled.

EXPERIMENTAL PROTOCOL

A wide range of coronary blood flow rates was tested in each animal by adjusting the pump rate. For each flow rate, the radiolabelled microspheres and subsequently the echocardiographic contrast agent were injected proximally to the mixing chamber. Since echocardiographic contrast agents may transiently alter the physiology of the coronary circulation[12], the injection of microspheres always preceded the contrast agent. To test the injection-to-injection variability, two contrast injections were performed at every flow rate, with a least a 5 min interval between each. In order to minimize heart motion, the ventilator was turned off immediately before the echo contrast agent was injected.

TRANSMURAL BLOOD FLOW DISTRIBUTION BY MICROSPHERES

The methods used to measure the transmural distribution of myocardial blood flow have been previously described[13]. 1×10^5 radioactive microspheres (15 μm in diameter) were injected into the circumflex artery, proximal to the mixing chamber. Injections were performed at three to six different flow rates for each dog. The microspheres were labelled with ^{153}Gd, ^{113}Sn, ^{103}Ru, ^{56}Co, ^{51}Cr, or ^{46}Sc (DuPoint, New England Nuclear). At the end of the experiments, the animals were killed with i.v. potassium chloride; the heart was removed and Evans Blue dye was injected into the circumflex coronary artery to stain the perfused territory.

The heart was then fixed in 10% formalin and cut into five to six transverse slices (from the base to the apex); the stained myocardium in each slice was removed, divided into wedges and each wedge was then cut into an inner, middle and outer layer. The rest of the heart was also divided into small pieces. Each piece was weighed and then counted. The radioactivity was analysed with a two-channel gamma counter (LKB Ultrogamma, Mod 1280). The sample weights and counts were transferred to a computer (Hewlett Packard 1000) in order to obtain flow per gram of each piece. Coronary blood flow to the endo-, mid- and epicardial layers was measured according the equation $f = F \times c/D$, where F is the pump flow, c is the counts/gram of the endo-, mid- and epicardial layers in the central portion of the stained area and D is the total count for the whole heart, i.e. the dose of injected microspheres.

ECHOCARDIOGRAPHIC IMAGE ANALYSIS

Echocardiographic images were digitized off-line using an array processor-based system for medical image processing: Mipron (Kontron, Germany)[14]. A 256×256 pixel matrix with 8 bits of intensity range was used. Digitized end-diastolic images were stored on disk. Digitization included at least four beats preceding the appearance of myocardial contrast and lasted for 20 s. The endo- and epicardial edges of each image were delineated by the operator. The centre of gravity of the left ventricular cavity was automatically identified and a grid, centred on it, was created to divide the myocardium into 16 equal sectors and each sector into three layers of equal thickness, corresponding to endo-, mid- and epicardial myocardium (Fig. 1). In the sectors corresponding to the central portion of the circumflex perfusion territory, located posteriorly, mean videodensity was measured for each of the three myocardial layers. These values were transferred to a personal computer (Macintosh, Apple) for subsequent analysis.

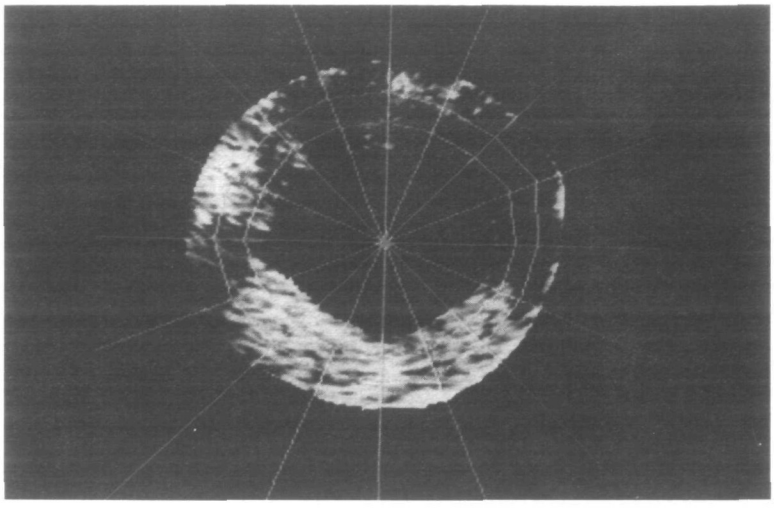

Figure 1 Computerized analysis of a contrast echocardiographic image in the short axis view. The left ventricular cavity and the structures outside the epicardium have been set to zero gray level. The ventricular myocardium is automatically divided into 16 sectors of equal angles and each sector into three layers of equal thickness, corresponding to the endo-, mid- and epicardial layers. The perfusion territory of the circumflex coronary artery, enhanced by the contrast effect, is included between 4 and 10 o'clock.

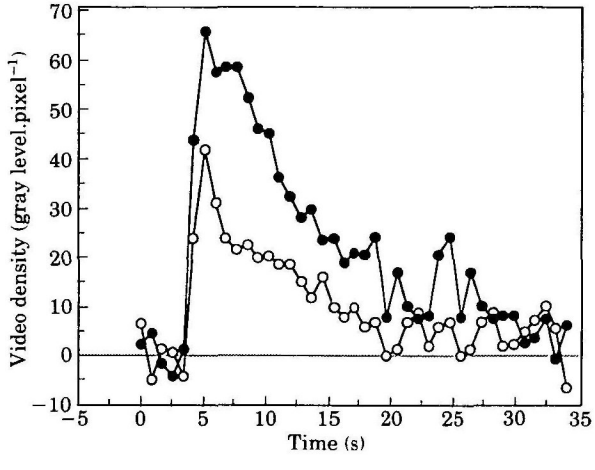

Figure 2 Myocardial contrast echo time-intensity curves corresponding to the subendocardial (white cicles) and subepicardial layers (black circles). Both videodensity (after background subtraction) and time are displayed in a linear scale.

ANALYSIS OF CURVES

Videodensity values of the entire wall, as well as the endo-, mid- and epicardial layers, were plotted against their corresponding times. Time-intensity curves were obtained as shown in Fig. 2 for the endo- and epicardial layers. These curves exhibited a rapid ascending phase, a peak and a slower descending phase. Both the background and peak contrast intensity of each curve were measured. Visual examination revealed that the initial portion of the washout phase was linear. The fact that a rectilinear decay phase was obtained when data were plotted on a linear scale was interpreted as indirect evidence of the logarithmic compression which exists in commercial sector scanners[15]. After background subtraction, the relationship between videodensity and time was tested in the linear portion of the washout phase of the curves using the least square linear regression analysis. The washout slope was then derived from the regression equation. The endo/epicardial ratio of contrast washout slope and peak contrast intensity were obtained by dividing the corresponding values.

STATISTICAL ANALYSIS

The least square linear regression analysis was used to test the relationship between videodensity and time in the washout phase of the curves. The same test was used to study the correlation between the slope of myocardial echo contrast washout and regional coronary blood flow, as well as to evaluate the relationship between the endo/epicardial ratio, as found with microspheres and with contrast echocardiography.

Results

CORONARY BLOOD FLOW AND ITS TRANSMURAL DISTRIBUTION BY MICROSPHERES

Data were collected at 21 different levels of coronary blood flow, representing three to six levels in each dog. A wide range of coronary flow rates was examined: from 0·76 ml . min^{-1} . g^{-1} up to 12·89 ml . min^{-1} . g^{-1} of myocardium. A wide range of coronary blood pressures was also explored: systolic blood pressure ranged from 35 to 153 mmHg and diastolic from 23–114 mmHg. The analysis of coronary blood flow distribution by radionuclide labelled microspheres also showed wide ranges in transmural blood flow distribution; the endo/epicardial flow ratio, as derived from microspheres, ranged from 0·53 to 1·73. As expected, a relative subendocardial

underperfusion occurred at low rates of coronary flow. Thus, a linear correlation existed between coronary blood flow and its transmural (endo/epicardial) distribution (correlation coefficient r = 0·60).

STUDY FEASIBILITY

Because two injections of contrast agent were performed at each of the 21 flow rates, a total of 42 contrast injections were given. Three contrast injections were not suitable for analysis owing to the poor quality of the echocardiographic images. A total of 39 injections were therefore analysed. The slope of the initial washout phase and peak contrast effect of myocardial time–intensity curves were derived from each of the 39 curves.

CORONARY BLOOD FLOW AND ITS TRANSMURAL DISTRIBUTION BY CONTRAST ECHOCARDIOGRAPHY

As previously shown[5], the slope of initial myocardial echo contrast washout was higher at higher coronary flow rates, showing a direct and close correlation with coronary blood flow (r = 0·85, Fig. 3). A positive correlation was also found between the slope of initial contrast washout of the mid-myocardial layer and the corresponding regional coronary blood flow, as found with microspheres (r = 0·71, Fig. 4). The accuracy of this correlation declined when the washout for the endo- and epicardial layers were compared with corresponding regional flow rates, the correlation coefficients r being 0·56 and 0·58, respectively (Figs 5, 6). Finally, the endo/epi ratio of initial contrast washout by echocardiography did not show any significant correlation with either coronary blood flow or its transmural distribution measured with radiolabelled microspheres (Fig. 7). Peak contrast intensity did not show any significant correlation with the blood flow of the entire wall or of any ventricular layer.

VARIABILITY OF CONTRAST ECHO MEASUREMENTS

To evaluate the injection-to-injection variability of the contrast echo measurements, the difference in values from two consecutive injections, performed at the same level of coronary flow, was calculated. The percentage error was considerable for the entire ventricular wall, but rose markedly when each of the different layers of the myocardium was considered. In fact, the error in washout slope was 27% for the entire ventricular wall, and 45%, 61% and 33% for the endo-, mid- and epicardial layers, respectively.

Discussion

This study shows that the washout of sonicated iopamidol from different myocardial layers corresponds only weakly with regional coronary blood flow, as measured with microspheres. This occurs despite the good correlation between myocardial washout from the entire ventricular wall and corresponding coronary blood flow. Furthermore, this study shows that the measurement of regional washout from each of the three myocardial layers is affected by an unacceptably high injection-to-injection

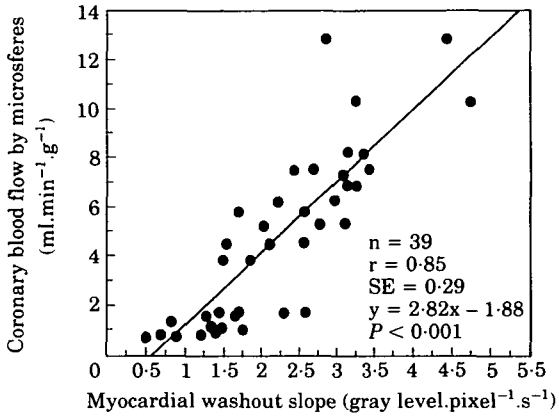

Figure 3 Correlation between initial slope of myocardial echo contrast washout of the entire ventricular wall and coronary blood flow. n = number of observations, r = correlation coefficient, SE = standard error of the estimate.

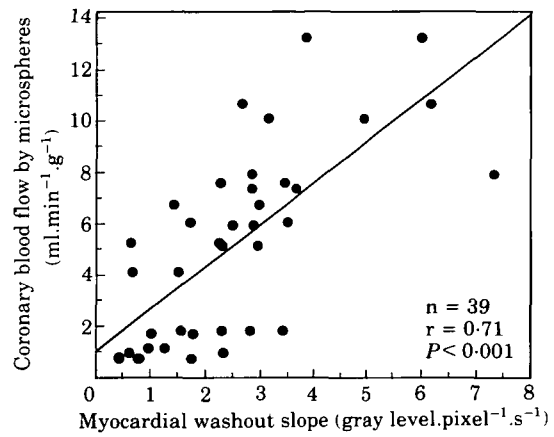

Figure 4 Correlation between initial slope of myocardial echo contrast washout of the midmyocardial layer and corresponding coronary blood flow.

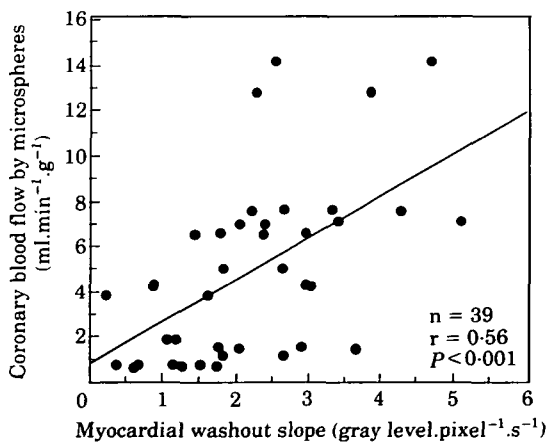

Figure 5 Correlation between initial slope of myocardial contrast washout of the subendocardial layer and corresponding coronary blood flow.

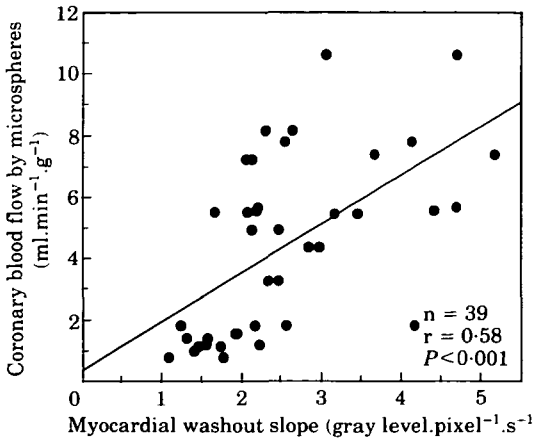

Figure 6 Correlation between initial washout slope of the subepicardial layer and corresponding blood flow.

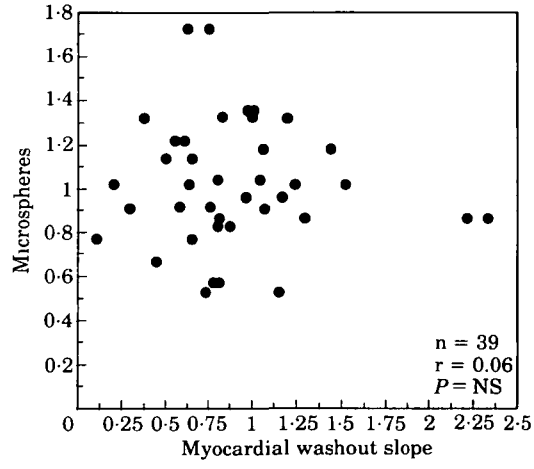

Figure 7 Relationship between the endo/epicardial ratio of myocardial echo contrast washout and the endo/epi ratio obtained by radionuclide labelled microspheres.

variability. Thus, the transmural distribution of contrast washout is unable to predict the transmural distribution of coronary blood flow, as independently assessed by radionuclide labelled microspheres.

POSSIBLE INTERPRETATIONS

The inability of myocardial contrast echocardiography to demonstrate the transmural distribution of coronary blood flow has been attributed by Kaul *et al.* to several factors[8]. They suggested that contrast microbubbles reflect a different flow from that of radionuclide labelled microspheres: the latter reflect arteriolar blood flow, whereas contrast microbubbles, being smaller than microspheres, may also reflect capillary and venular flow. According to this hypothesis, contrast microbubbles may cross to regions of the myocardium which are different from those that they initially entered, and thus a contamination of contrast echo images may occur. In addition, contrast microbubbles would reflect ultrasonic waves in multiple directions, behaving like scatterer reflectors. Thus, each bubble would receive ultrasonic energy not only from the transducer, but also from the surrounding bubbles, independently of their transmural distribution.

An additional attractive interpretation deals with flow–volume relationships[8]. The kinetics of a tracer is related to both flow and volume of distribution of the tracer. If the volume is constant, the transit of the tracer reflects flow. If the volume changes, transit of the tracer can no longer be related to flow. A reduction in intramyocardial blood volume is likely to occur in the subendocardial layers during myocardial ischaemia, because of the fall in coronary perfusion pressure. This regional reduction in blood volume might shorten the transit time of a tracer, thus masking the prolongation of contrast transit time expected to occur in the subendocardial layer during acute myocardial ischaemia. To minimize the effects of changes in intramyocardial blood volume on myocardial washout rate of the contrast agent, all contrast injections were performed in this study after abolishing coronary autoregulation by intracoronary adenosine infusion. However, even a continuous infusion of adenosine does not ensure constant intramyocardial blood volume, because different degrees of perfusion pressure and intraventricular pressure can influence vascular capacitance[16,17].

In our opinion an additional mechanism which may explain the inability of myocardial contrast echocardiography to predict the transmural distribution of coronary blood flow may be related to the lack of reproducibility of contrast echo measurements. This study shows that the reproducibility of these measurements declines as the region of interest becomes smaller. In fact, the analysis of the same echocardiographic images showed a greater reproducibility of measurements when the entire ventricular wall rather than the different myocardial layers were sampled. This decline in reproducibility when thin myocardial layers are interrogated is likely to reflect a reduction in the signal/noise ratio of the data collected. The noise is a random phenomenon, which declines with signal averaging as when a large region of interest is sampled. Recent studies have also demonstrated that an increase in surrounding pressure enhances the decay of contrast microbubbles, irrespective of the flow rate[18,19]. In the coronary circulation, contrast microbubbles are exposed not only to coronary pressure, but also to the intramural pressure, which is greater in the subendocardial than in the subepicardial layers[20]. This heterogeneity in intramural pressure might have contributed to the inaccuracy of the regional blood flow quantitation. Furthermore, it should be stressed that these data have been calculated using the principles of indicator–dilution theory, whereas the contrast agent used in this study diverges from an ideal flow tracer. Finally, conventional scanners cannot obtain a linear relationship between the concentration of the tracer (contrast microbubbles) and the intensity signal, a prerequisite for the measurement of blood flow by indicator dilution.

COMPARISON WITH THE LITERATURE

These results are at variance with those reported in previous studies. Specifically, the transmural distribution

of blood flow was assessed by myocardial contrast echocardiography in dogs with circumflex coronary stenosis[6]. In the regions perfused by the stenotic vessel, the endo/epicardial flow ratio, as derived with microspheres, significantly decreased after dipyridamole, as did the endo/epicardial ratio of areas under the myocardial time-intensity curve. In the present study all contrast injections were performed during intracoronary infusion of adenosine, which is the mediator of dipyridamole[21], and no significant correlation was found between transmural distribution of contrast intensity or of contrast washout and transmural distribution of coronary flow. The results of the present study also diverge from those reported in patients with coronary artery disease, where a reduction in the endo/epicardial ratio of peak contrast intensity was observed during acute myocardial ischaemia induced by rapid atrial pacing[7]. The physiological heterogeneity of the transmural distribution of coronary blood flow during the cardiac cycle has been previously evaluated by contrast echocardiography in our laboratory[22]. The increment in myocardial videodensity was measured in the frame where the echo contrast effect first appeared: the increment in videodensity was greater in the endocardial layers when the contrast effect initially appeared in diastole; conversely, the increment in videodensity was greater in the epicardial layers when the contrast effect initially appeared in systole. However, in the present study, the sampling of the echocardiographic images was limited to end-diastole, and myocardial contrast washout, instead of appearance, was evaluated.

Our results are in agreement with a recent experimental study, in which the transmural distribution of flow was measured using radiolabelled microspheres and data compared with those obtained by two different echo contrast agents: sonicated albumin and hand-agitated microbubbles[8]. Despite the previous demonstration that mean transmural blood flow may be assessed by myocardial contrast echocardiography[4], the authors found that the ratios of the parameters derived from the endo- and epicardial layers during myocardial contrast echocardiography correlated poorly with the microsphere derived endo/epi flow ratio. These results were not influenced by either the location of the regions of interest selected for the analysis or the size of contrast microbubbles.

Conclusions

This study confirms that the overall coronary blood flow through the full thickness of the ventricular wall may be quantified by myocardial contrast echocardiography. However, this study also shows that myocardial contrast echocardiography with sonicated iopamidol is unable to measure the transmural distribution of coronary blood flow. In the future this goal may become possible using different contrast agents (closer in characteristics to a flow tracer) and different scanners (capable of measuring the concentration of contrast microbubbles).

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