Visualization and quantification of myocardial mass at risk using three-dimensional contrast echocardiography


Medical University of Łódź, Łódź, Poland
Thoraxcentre, University Hospital Rotterdam—Dijkzigt, Rotterdam, The Netherlands
Erasmus University, Rotterdam, The Netherlands
Andaris Ltd., Nottingham, UK

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Abstract

Objective: Three-dimensional echocardiographic assessment of myocardial ischemia using contrast echocardiography has been hampered by limitations of available contrast agents and analytic software. In the study presented, a three-dimensional perfusion imaging method was evaluated in the porcine model of myocardial ischemia using a novel contrast agent. Methods: Three-dimensional echocardiography was performed in eight open-chested pigs before, during and after left anterior descending (six animals) or circumflex (two animals) coronary artery occlusion. The intramyocardial contrast effect was obtained by left atrial injection of Myomap, a deposit contrast agent. Results: Myocardial opacification was visible in all studies and retained in all three-dimensional datasets. Three-dimensional intensity analysis demonstrated a significant difference, exceeding 20 intensity units in every animal (in 127-level scale), between perfused and non-perfused myocardium. Reperfusion followed by contrast reinjection resulted in homogenous myocardial enhancement. Myocardial mass at risk was clearly delineated in all studies and measured with a mean error of $0.1 \pm 2.0$ g against real mass ($p = \text{non-significant}$). Spatial extent of ischemia could be displayed in volume-rendered reconstruction of separate perfusion territories. Conclusions: Quantitative analysis of myocardial contrast enhancement from three-dimensional datasets is feasible and allows accurate measurement of myocardial mass at risk. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Ischemia; Ultrasound; Three-dimensional reconstruction; Porcine; Contrast media; Regional blood flow; Ischemic mass quantification

1. Introduction

Myocardial perfusion abnormalities can be evaluated using echocardiographic contrast imaging [1–4]. Currently used contrast agents behave as flow tracers, providing brief, dynamic myocardial enhancement. Their usefulness is hampered due to a short temporal window for data acquisition, signal attenuation and sensitivity to ultrasound pressure. These drawbacks precluded efficient three-dimensional echocardiographic reconstruction of myocardial perfusion territories. Recently, a novel deposit echocardiographic contrast agent—Myomap (Andaris, Nottingham, UK) became available. Contrary to previously used agents, high pressure stability of Myomap microbubbles enables a prolonged myocardial intensity enhancement, sufficiently long for three-dimensional echocardiographic data acquisition [5]. A three-dimensional contrast ech-
2. Methods

2.1. The animal study design

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and under the regulations of the animal care committee of the Erasmus University, Rotterdam. The experiments were conducted on eight Yorkshire open-chested pigs, weighing 21.5±31 kg (mean 25.8±3.1). Animals were sedated with 25 mg/kg intramuscular ketamine-HCl, followed by 20 mg/kg intravenous pentobarbital. During the experiments a continuous infusion of pentobarbital (10 mg/kg/h) was given. Heart rate, electrocardiograms (ECG), aortic pressures, cardiac output (electromagnetic flow meter was used) as well as left ventricular pressures and derivatives were continuously monitored. Before the contrast study, indomethacin (0.2 mg/kg body-weight) was given intravenously. As soon as the hemodynamic parameters stabilized after preparation of the animal, baseline three-dimensional echocardiographic acquisition was performed. Subsequently, left anterior descending coronary artery (LAD) was occluded in six pigs and circumflex artery (CX) in two. After one minute, 20 ml of Myomap™ was injected through the left atrial catheter. One minute after injection the image acquisition was repeated. Arterial clamp was released after 10 min and the injection of 20 ml Myomap™ was repeated during the reperfusion, followed by another three-dimensional echocardiographic image acquisition. Left ventricular mass (excluding the papillary muscles) was weighed at the animal sacrifice. Myocardial mass at risk was delineated using a postmortem intracoronary dye (methylthionine) injection distally to the occluder, separately excised and weighed.

2.2. Deposit contrast agent

Myomap™ (Andaris) is a contrast agent consisting of air filled microcapsules, formed by spraying 20% human albumin solution. The mean diameter of the contrast microspheres used in our study was 10 µm, with less than 0.5% exceeding 20 µm. The walls of the microcapsules are composed of cross-linked human serum albumin and are 1 µm thick. The agent is prepared by reconstitution of a dry powder in Tween-80 solution, resulting in concentration of 1.5·10^7 microcapsules/ml. The volume of Myomap™ used in our study was chosen according to the results of three preceding dose-finding experiments (data on file, Andaris). In those studies the dose of 20 ml provided optimal homogenous myocardial opacification without the saturation of greyscale.

2.3. Three-dimensional echocardiography

Echocardiographic images were acquired using a 5 MHz transesophageal multiplane probe placed over the right ventricular wall. Constant settings of two-dimensional images, optimized for the visualization of left ventricular endocardium with a linear postprocessing curve were used. ECG-gated images for three-dimensional reconstruction were acquired with a rotational technique at 2° interval using a three-dimensional echocardiographic system (Echo-Scan 3.0, TomTec, Munich, Germany) as described in detail before [6]. Original two-dimensional images were videotaped and subsequently used to measure segmental wall thickening before and after contrast injection (Table 1). Myocardial thickness was measured using electronic calipers of a dedicated off-line digitizer workstation.

2.4. Image display and analysis

Post-processed three-dimensional echocardiographic datasets were used for reconstruction of parallel cardiac cross-sections perpendicular to the selected left ventricular long axis (paraplane echocardiography). Myocardial volume was covered with eight equidistant slices. Epicardial and endocardial contours of perfused and non-perfused left ventricular wall were manually traced in reconstructed cutplanes. Myocardial volume was calculated using a modified Simpson’s method, according to a quantification protocol validated at our institution [7]. Muscle mass was calculated as the product of volume and muscle density (1.05 g/cm³).

In an alternative display modality, data were used to generate a solid-looking representation of myocardium in three-dimensional perspective (volume rendering). The rendering of selected portions of the dataset (“electronic vivisection”) was performed to create spatial reconstruction of perfused and non-perfused parts of the myocardium (Fig. 1).

Myocardial contrast enhancement was quantitatively determined by intensity analysis (in 1–127 scale) within volumetric cubic samples of 4–6 mm side length taken from perfused and non-perfused myocardium.

2.5. Statistical analysis

Numerical data are expressed as mean±standard deviation. Linear regression and Bland–Altman analysis [8] was used to determine the agreement between myocardial mass at risk calculated from three-dimensional echocardiogram and gross specimen weight. Comparison of data obtained at different stages of the experiment was made with analysis of variance using the Bonferroni method. Differences were considered significant at p<0.05. Measurement
Table 1

Hemodynamic effects of Myomap™ injections

<table>
<thead>
<tr>
<th>Hemodynamics</th>
<th>Baseline</th>
<th>Pre-inj. 1</th>
<th>Post-inj. 1</th>
<th>Pre-inj. 2</th>
<th>Post-inj. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>108.8±19.9</td>
<td>101.8±19.3</td>
<td>102.8±18.8</td>
<td>100.5±18.0</td>
<td>99.0±19.7</td>
</tr>
<tr>
<td>SAP (mmHg)</td>
<td>107.9±8.4</td>
<td>110.5±8.1</td>
<td>109.1±10.7</td>
<td>110.7±9.1</td>
<td>107.8±12.6</td>
</tr>
<tr>
<td>DAP (mmHg)</td>
<td>76.4±9.3</td>
<td>78.8±10.0</td>
<td>80.2±12.2</td>
<td>80.1±11.0</td>
<td>77.8±12.5</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>85.9±9.1</td>
<td>90.1±10.0</td>
<td>91.0±12.2</td>
<td>91.0±10.7</td>
<td>88.8±12.9</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>8.0±1.1*</td>
<td>9.2±1.5</td>
<td>9.3±1.5</td>
<td>8.9±2.2</td>
<td>9.0±2.8</td>
</tr>
<tr>
<td>LV dP/dt max (mmHg/s)</td>
<td>1906±547</td>
<td>1663±319</td>
<td>1688±353</td>
<td>1567±298</td>
<td>1563±302</td>
</tr>
<tr>
<td>Cardiac output (l/min)</td>
<td>2.9±1.3</td>
<td>2.8±1.0</td>
<td>2.5±1.0</td>
<td>2.6±0.9</td>
<td>2.4±0.9</td>
</tr>
<tr>
<td>%WTh of perfused segments</td>
<td>23.7±6.5%</td>
<td>25.0±7.6%</td>
<td>25.7±8.0%</td>
<td>25.2±6.2%</td>
<td>25.5±3.8%</td>
</tr>
</tbody>
</table>

Hemodynamic variables obtained from continuous monitoring during the experiments as well as segmental myocardial function expressed as %wall thickening remain unchanged during Myomap™ dosing (after the preparation of the animals).

*p<0.001 between baseline and pre-injection 1.

Other differences, calculated between baseline and pre-inj. 1, between pre-inj. 1 and post-inj. 1 as well as between pre-inj. 2 and post-inj. 2, were not significant.

Abbreviations: Pre-inj. 1, 1 min before the first injection of Quantison, during coronary occlusion; Post-inj. 1, 2 min after the first injection of Quantison, during coronary occlusion; Pre-inj. 2, 1 min before the second injection of Quantison, during reperfusion; Post-inj. 2, 2 min after the second injection of Quantison, during reperfusion; SAP, systolic aortic pressure; DAP, diastolic aortic pressure; MAP, mean aortic pressure; LVEDP, left ventricular end-diastolic pressure; LV dP/dt max, maximum left ventricular first derivative of pressure; %WTh, percent wall thickening = (end systolic thickness−end diastolic thickness)/end diastolic thickness × 100%.

reproducibility was expressed as coefficients of variability [8].

3. Results

3.1. Effectiveness of the deposit contrast agent

The protocol was completed in all animals. There were no significant hemodynamic adverse effects related to Myomap™ injection. The thickening of perfused myocardium was unchanged by the contrast agent as shown in Table 1.

After left atrial injection, Myomap™ caused a visible increase in myocardial echo intensity, limited to the perfused part of the muscle (Figs. 1 and 2). During reperfusion, intense opacification of entire myocardium was observed in all pigs, persisting until the end of the

Fig. 1. Visualization of perfusion defects using Myomap™ during the occlusion of left anterior descending artery (LAD, panels A–C) and left circumflex artery (CX, panels D–F). The borders of myocardial mass at risk (arrows) are clearly delineated in two-dimensional short-axis views of the left ventricle long after the disappearance of cavity opacification (A,D). Volume rendered reconstruction of perfused (C,F) and non-perfused (B,E) parts of the muscle displays extracted complementary parts of the myocardium in three-dimensional perspective.
Fig. 2. Three-dimensional echocardiographic analysis of regional perfusion. Anyplane views are reconstructed after contrast agent injection. Greyscale histograms are derived from cubic labels placed in the muscle (black squares). Imaging during the occlusion of left anterior descending artery (A,B) demonstrates myocardial enhancement except for the antero–septo–apical region (short arrows). Greyscale distribution histograms within perfused (A) and non-perfused (B) muscle confirm a higher intensity in perfused, contrast-enhanced areas. Data obtained after the release of occlusion and contrast reinjection (C) demonstrate a marked hyperemic response (rightward shift of greyscale histogram).

experiment (30 min), long after the enhancement had disappeared from the ventricular cavity. The reperfused area at risk could no longer be delineated after contrast reinjection.

3.2. Three-dimensional contrast echocardiography

Three-dimensional reconstruction of left ventricular myocardium was feasible in all studies. Acquisition time ranged 5–8 min, whereas data processing and analysis never exceeded 90 min for one experiment. Contrast enhancement of the perfused muscle was visible in the computer-reconstructed cross-sections (paraplane echocardiography). Echo intensity difference between perfused and non-perfused muscle (similar for LAD and CX zone) was confirmed by three-dimensional intensitometry as shown in Fig. 2. In each animal, a significant myocardial signal enhancement exceeding 20 greyscale units was seen in the perfused area after Myomap™ injection ($p<0.001$). The differences in echo intensity parameters from non-enhanced and enhanced muscle are summarized in Table 2. The reproducibility of the method was good (mean difference between the measurements repeated no sooner than after 1 month was $0.9\pm 3.7$). The perfused and non-perfused part of the muscle could be separately extracted and displayed as a volume-rendered image in every study, providing direct information about the spatial relations of the myocardial area at risk (Fig. 1).

Table 2

<table>
<thead>
<tr>
<th></th>
<th>Before contrast injection</th>
<th>Diastole</th>
<th>Systole</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1</strong> Left ventricular blood pool</td>
<td>9.8±7.2*</td>
<td>12.2±10.3*</td>
<td></td>
</tr>
<tr>
<td><strong>2</strong> Basal septum</td>
<td>32.6±8.7</td>
<td>39.7±12.4</td>
<td></td>
</tr>
<tr>
<td><strong>3</strong> Non-perfused segment</td>
<td>27.8±7.6</td>
<td>27.0±8.4</td>
<td></td>
</tr>
<tr>
<td><strong>4</strong> Reperfused segment</td>
<td>55.3±11.8</td>
<td>65.5±15.9*</td>
<td></td>
</tr>
<tr>
<td><strong>5</strong> Basal septum (perfused)</td>
<td>62.3±11.4</td>
<td>62.1±9.7*</td>
<td></td>
</tr>
</tbody>
</table>

Perfused segments demonstrate a major increase in signal intensity after contrast injection, consistent with the visual findings. Mass at risk (rows 3,4) remains contrast-free during the occlusion but has similar signal intensity as other segments after the second dose of contrast during reperfusion. Segments referred to in rows 3 and 4 are: middle anterior septum in six pigs with left anterior descending occlusion and middle posterior wall in two pigs with circumflex occlusion. Significance values are calculated according to the Bonferroni method.

* significant difference ($p<0.05$) as compared to values 2,3,4,5.

* significant difference ($p<0.05$) as compared to values 2 and 3.
3.3. Myocardial mass at risk quantification

Weighed total myocardial mass ranged from 64.1–102.3 g (mean 79.5±13.2). Mass at risk calculation was feasible in all three-dimensional datasets during both LAD and CX occlusion. Ischemic muscle mass calculated from three-dimensional echocardiography (18.1–37.9 g, mean 24.0±6.3 g) agreed closely with real weighed values: 17.0–36.7 g, mean 24.1±6.0 g; mean difference 0.10±2.03 g, \( p = \text{non-significant (NS)} \) as shown in Fig. 3. The masses obtained by both methods were closely correlated (real mass=0.91×three-dimensional echocardiographic estimate+2.3 g, \( p < 0.001, r = 0.947, \) standard error of the estimate 2.1 g). Calculated percent of myocardial mass supplied by the occluded artery was in close agreement with its real value (30.0±3.9% and 30.3±4.1% respectively, mean difference 0.3±3.0%, \( p = \text{NS} \)). Reproducibility of measurements was high (intra- and inter-observer variability coefficient 4.6% and 6.2% respectively).

4. Discussion

The results of this study show that myocardial mass at risk can be visualized and quantitated using a deposit ultrasound contrast agent and three-dimensional echocardiography. The feasibility of signal intensity quantification within three-dimensional datasets has been demonstrated for the first time.

Previous contrast echocardiographic studies assessing myocardial perfusion relied on the analysis of two-dimensional muscle cross-sections. However, complex spatial distribution of perfusion warrants examination in three dimensions. Three-dimensional echocardiographic studies are performed in humans using transthoracic and transesophageal approaches [9,10], improving the perception of complex geometry of the heart [11] and providing very accurate quantitative data [12]. It has been demonstrated that ischemic myocardial mass can be quantitated using three-dimensional measurements of the volume of abnormally contracting muscle [13]. The lack of appropriate software tools and stable signal enhancers has hampered progress in three-dimensional contrast echocardiography. Previous study from our center demonstrated the feasibility of three-dimensional reconstruction of sequentially video-registered cutplanes with custom-designed software [14]. Initial experience with short-living flow tracers—agitated saline [15] or albumin perfluoropropane-filled bubble contrast agents was reported [16,17] demonstrating qualitative myocardial enhancement and feasibility of spatial reconstruction of perfusion defects. However, a short temporal window available for image acquisition is a limiting factor for the size of the examined zone and the resolution of imaging. The acquisition of data for three-dimensional reconstruction of myocardial perfusion, usually consuming a few minutes, necessitates a continuous infusion rather than a single dose of such agents.

In our study, left-sided injections of Myomap™ provide pronounced and long-lasting myocardial opacification, persisting long after blood pool enhancement had disappeared. Thereby, the signal attenuation by cavity contrast is eliminated, facilitating precise delineation and accurate quantification of ischemic muscle mass, both in
LAD and CX beds. This effect cannot be obtained with currently used flow tracers unless selective intracoronary injections are used. Another benefit of an agent with a resistant microcapsule shell is pressure insensitivity. Previously used contrast agents are destroyed by diagnostic acoustic pressures. This results in unreliable myocardial opacification, especially if its longer persistence is required as is in the case of three-dimensional echocardiography. Conversely, a stable shell allows efficient imaging even in continuous mode, facilitating simultaneous evaluation of blood supply and wall motion. Although recently available real-time three-dimensional echocardiography allows rapid data acquisition [18,19], the attenuation and pressure-sensitivity problem remain.

A distinct border between perfused and non-perfused muscle was seen in all coronary occlusion studies after the first dose of Myomap™ whereas the contrast reinjection after the release of arterial clamp resulted in complete, homogenous opacification of reperfused myocardium. Prolonged myocardial enhancement creates a sufficient temporal window for high quality three-dimensional reconstruction after a single bolus of the agent. Thus, the mass of ischemic myocardium can be reproducibly assessed, providing excellent agreement with real values as shown in our study. This is concordant with the results of a recently published study [20], where ischemic and infarcted myocardial masses were closely correlated with autoradiographic and TTC staining data in a canine model. The presence of reactive hyperemia was not helpful for the delineation of the reperfused area due to the accumulation of myocardial enhancement caused by two consecutive injections of the deposit agent in the perfused part of myocardium. Therefore, the mass at risk could not be delineated at this stage. Recent reports describe the usefulness of other methods, such as gadoteridol-enhanced magnetic resonance imaging for the quantification of infarcted mass [21] and risk stratification [22] after myocardial infarction. This approach is also effective for the visualization of infarction after the reperfusion, including the differentiation of reflow pattern by the analysis of hyper- and hypo-enhanced areas. In our experimental protocol the duration of ischemia was too brief to result in persistent microvascular damage and myocardocyte necrosis, which is confirmed by the restoration of contractility in reperfused areas. The study by Linka et al. [20] suggests that three-dimensional contrast echocardiography may also be used for the quantification of infarcted mass although further validation in humans is necessary.

Myomap™ with its relatively large and thick-walled bubble is a deposit agent, resembling radiolabelled microspheres. In theory, such a tracer should be completely retained in the arterioles of myocardium. The presence of smaller microcapsules in the Myomap™ preparation may result in minor flow tracer properties but this is probably negligible, particularly if more delayed images are analyzed. Echo intensity should thus reflect relative flow to different regions of myocardium. This was the rationale behind our attempt to validate the quantification of the signal intensity in three-dimensional samples with new, custom-written software. The utility of three-dimensional intensitometry for more detailed quantitation of local coronary flow in the presence of coronary artery stenosis remains yet to be investigated. Our study demonstrates the feasibility of echo intensity quantification within three-dimensional datasets, the analytic modality never described before. The possibility to assess echo intensity within spatial objects is unique to the three-dimensional technique and can extend the evaluation of contrast enhancement beyond planar cutplanes. Calculated intensity differences between bloodpool, non-enhanced myocardium and opacified myocardium were in agreement with visual assessment of contrast. In our study, three-dimensional intensity analysis confirmed that the visible myocardial signal enhancement always exceeded 20 units (in 127-level scale), which should be easily discernible in transthoracic studies. In future, this approach can also be used for integrated backscatter analysis and automated image segmentation techniques, particularly in future real-time systems. Myocardial contrast enhancement is reported to be more pronounced when harmonic imaging is used [23]. Further possible developments can include three-dimensional reconstruction of the second harmonic signal although the optimal resonant frequency of the Myomap™ is lower than echocardiographic diagnostic frequencies.

The myocardial opacification pattern was helpful for the extraction and reconstruction of the perfusion zones. The dynamic volume-rendered display (Fig. 1) was obtained from all datasets, reproducing the shape of area at risk in gross specimen. However, contrast enhancement cannot be assessed in this display mode because the shading algorithms, used to create the illusion of depth, change the brightness of an object [24].

If human perfusion territories could be studied using a similar approach, the technique may find a clinical application in patients undergoing cardiac catheterization. Left ventricular injection of Myomap™ could be given when the invasive procedure is completed. Three-dimensional study of mass at risk might thus be delayed until the patient returns to the ward. Contrary to Myomap™, previously used intravenous contrast agents provide short-living myocardial opacification, are sensitive to destruction by ultrasound pressures and attenuation-related problems are overwhelming.

Similarly, in acute coronary syndromes, the technique may detect the area at risk if contrast is injected prior to reperfusion. Homogenous myocardial opacification would be suggestive of successful reflow, e.g., after PTCA or thrombolysis. Analogous clinical application can be proposed in the setting of the operating theatre. A single left ventricular injection of the contrast, providing long-lasting myocardial opacification suitable for three-dimensional analysis, might be used to assess the functional effects of
coronary bypass surgery. Since silent myocardial infarction occurs in 10% of CABG patients, such information can be useful for immediate clinical management.

4.1. Limitations

Myomap™ did not cause any hemodynamic deterioration or detectable decrease of segmental thickening in our experiment. However, considering that the left-sided injection of a deposit agent implies microembolization of a number of small vessels, particular interest in safety arises. It is known from studies using radiolabelled microspheres of 15 μm that a dose of 5·10^7/g of tissue is needed to arrest the heart [25]. To our knowledge, no toxicity data are available for radiolabelled microspheres with sizes comparable to the microcapsules used in the present study but safe left atrial administration of 22·10^6 microspheres of 7–10 μm diameter was reported [26]. Although the safety records from our study are encouraging, extensive studies are needed before the agent can be clinically applied.

Myomap™ is especially designed for left-sided injections. An intravenous injection will not result in transpulmonary passage and myocardial enhancement. Therefore, proposed clinical applications are complementary to invasive or surgical diagnostic procedures. Although the data in our study were obtained with epicardial acquisition, the intensity of opacification is expected to be easily detectable in transthoracic or transesophageal studies. We have used only a short period of ischemia and therefore the extrapolation of results to human myocardial infarction must be cautious. However, the experimental data from an experimental study with Myomap™ in an acute infarction setting [20] support the validity of proposed clinical applications.

Despite the proven accuracy in quantification, three-dimensional echocardiography still suffers from several technical limitations. Reconstruction is performed off-line and final spatial and temporal resolution is lower compared to two-dimensional echocardiography. Software procedures, facilitating the assessment of contrast enhancement, segmentation of perfusion areas and quantitative analysis have yet to be created. Moreover, the impact of data interpolation during three-dimensional reconstruction upon final greyscale values is not known.

The method we used for postmortem measurement of area at risk may overestimate the real size of the mass at risk due to the pressure gradient caused by the injection. However, the excellent correlation of three-dimensional and weighed mass at risk in our study is consistent with the values published by Linka et al. who used autoradiography as a reference (standard error of estimate 2.1 and 5.1 g, respectively). The results of Bland–Altman analysis were not shown in that report [20].

5. Conclusions

A deposit contrast agent, Myomap™, enables echocardiographic detection of perfusion abnormalities in animal model. Three-dimensional echocardiography permits precise spatial localization and highly accurate quantification of myocardial mass at risk. The feasibility of signal intensity analysis in three-dimensional echocardiographic datasets has also been demonstrated. The method may find a clinical application in the settings of cardiac catheterization laboratories and operating theatres.

References


