Myocardial Perfusion Abnormalities by Intravenous Administration of the Contrast Agent NC100100 in an Experimental Model of Coronary Artery Thrombosis and Reperfusion

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The aim of this study was to evaluate a second-generation echo contrast agent (NC100100) for the study of myocardial perfusion. In eight anesthetized open-chest dogs, this agent was injected intravenously under baseline conditions, during acute coronary thrombosis, and after reperfusion, using both fundamental (FI) and harmonic (HI) imaging, both continuous and intermittent imaging, and both ultrasound (US) and integrated backscatter (IBS) imaging. Contrast injections did not modify the hemodynamic parameters. With all imaging modalities, myocardial contrast enhancement (MCE) was higher with intermittent than with continuous imaging (134 vs 82 gray level/pixel using FI, P = 0.02; 62 vs 32 acoustic units using US HI, P = 0.02; and 52 vs 12 dB using IBS, P = 0.05). MCE equally increased using either US or IBS imaging. The accuracy of MCE in detecting perfusion defects during coronary occlusion and myocardial reperfusion after thrombolysis was very good (sensitivity and specificity = 93% and 95% and 89% and 93%, respectively). The extent of myocardial perfusion defects by echo contrast showed a closer correlation with microspheres using HI (r = 0.82) than FI (r = 0.53). Thus, the intravenous administration of NC100100 during intermittent HI allows myocardial perfusion abnormalities to be accurately detected during acute myocardial infarction. (ECHOCARDIOGRAPHY, Volume 15, No. 8, Part 1, November 1998)

myocardial perfusion, contrast echocardiography, experimental, myocardial infarction, harmonic imaging

In acute myocardial infarction, contrast echocardiography can provide useful information regarding the extent of risk area,[1-3] the occurrence of myocardial reperfusion,[4,5] the presence of collateral circulation[6,7] and of myocardial viability,[8-11] the no-reflow phenomenon,[4,5,12,13] and the late recovery of left ventricular (LV) function.[4,5,12] A myocardial echo enhancement can be easily obtained after the intracoronary administration of contrast agents; however, this invasive approach has limited use in the clinical setting. Over the past few years, pharmaceutical companies, echo industries, and cardiologists have made an effort to achieve the goal of noninvasive myocardial perfusion assessment. Thus, new contrast agents have been produced that allow not only transpulmonary passage but also myocardial contrast enhancement (MCE) after an intravenous administration, including PESDA,[14] EchoGen,[3] FS069,[15-19] BR1,[20,21] Aerosomes (MRX

115),[22] Imagent (AF0150),[23,24] QW7437,[25] Quantison,[26] and others.

A further increase in MCE can be obtained by a method based on emission of harmonics by resonating microbubbles,[23,27,28] as well as further enhancement by the use of intermittent imaging,[29] likely due to less destruction of microbubbles by ultrasound.[29,30] Finally, an improvement in myocardial perfusion evaluation might be obtained by the analysis of the native ultrasound signal, namely integrated backscatter (IBS), which has been shown to be useful in ultrasound tissue characterization.[31,32]

The aim of this study was to evaluate a new second-generation contrast agent (NC100100, Nycomed Imaging AS, Norway) for MCE in an experimental model of acute coronary artery occlusion due to coronary thrombosis and reperfusion. The specific purposes of the study were to evaluate the hemodynamic effects of this agent, to investigate the influence of different imaging modalities on MCE, and to test the ability to detect both perfusion defects during coronary occlusion and myocardial reperfusion after thrombolysis.

Methods

Experimental Animal Preparation

The protocol conformed to the "Position of the American Heart Association on Research Animal Use." The study was performed in eight adult mongrel dogs of either sex, weighting 17–23 kg. The animals were premedicated with morphine sulfate (2.5 mg/kg body weight SC), anesthetized with alpha-chloralose (100 mg/kg IV), intubated, and ventilated with room air by a positive-pressure respirator (Harvard Apparatus model 613). Anesthesia was maintained by the supplemental injections of alpha-chloralose (500 mg/hr). Throughout the experiments, arterial oxygen tension, carbon dioxide tension, and acid-base balance were maintained within physiological ranges by modifying the respirator rate, by increasing oxygen tension, or by administering sodium bicarbonate.

The dogs were studied in a slight right lateral decubitus position. A left thoracotomy was performed at the fourth intercostal space, the pericardium was excised and the heart was suspended in a pericardial cradle. The left circumflex coronary artery was dissected free from surrounding tissues and an electromagnetic flowmeter was implanted around the vessel. A pressure-tip catheter (Millar 7F) was inserted into the abdominal aorta through the right femoral artery. A Swan-Ganz catheter was advanced from the femoral vein to the pulmonary artery. An intravenous cannula was inserted into a forelimb vein for contrast injections, and another cannula was inserted into the left atrial appendage for microspheres injections. Two electrocardiogram leads, aortic pressure, pulmonary arterial pressure, and coronary blood flow were recorded on paper before and during contrast injections. Cardiac output was measured before and after contrast injections performed at baseline, during coronary occlusion, and after reperfusion.

Echocardiographic Examination

Two-dimensional echocardiograms were obtained with an electronic sector scanner (model 2500 Hewlett-Packard). The transducer was placed on the anterior wall of the LV with the interposition of silicon rubber and was oriented so as to obtain a short-axis view of the LV; once a good image was obtained, the transducer was maintained in a fixed position by a mechanical arm.

In the first four dogs, a scanner operating in fundamental imaging at 3.5 MHz was used. During data acquisition, overall gain, logarithmic compression, and time gain compensation were kept constant. Contrast echo images were collected with both continuous and intermittent imaging (sampling one frame every two cardiac cycles at end-diastole). Echocardiographic images were recorded on Super VHS videotape for subsequent playback and analysis.

In the last four dogs, a scanner implemented with harmonic imaging[23,27,28] and operating at 1.8/3.6 MHz was used. During harmonic imaging, the power output was adjusted so as to reduce the mechanical index to 0.3. Again, overall gain, logarithmic compression, and time gain compensation were kept constant, and contrast echo images were collected with both

continuous and intermittent imaging. With harmonic imaging, data were also collected using both ultrasound (US) and integrated backscatter (IBS) imaging. Echocardiographic images were stored in a digital format on optical disk. Using continuous imaging, two cine-loops were acquired: one corresponding to baseline conditions (before contrast) and another to visually discernible myocardial contrast effect; each loop corresponded to 62 frames and to 2.48 seconds. Using intermittent imaging, a sequence of frames triggered on the end-diastole was acquired, starting a few seconds before contrast injection and sampling one every two cardiac cycles.

Echocardiographic Contrast Agent

NC100100 was the echo contrast agent used in all the experiments. This agent contains stabilized microbubbles of a perfluorocarbon gas, which has low solubility in blood. The microbubbles have a relatively narrow size distribution, with a mean diameter of 3–4 μm and an interquartile width of 1–3 μm. The contrast agent was injected intravenously, as a bolus, at the dose of 0.10 μl/kg of body weight for fundamental imaging and 0.03 μl/kg for harmonic imaging, followed by a saline flush. The volume of the contrast agent administered in each injection ranged from 0.06 to 0.4 μl. The injections of the agent were separated by an average time interval of 27 minutes. Examples of echocardiographic images obtained after intravenous injection of this agent using intermittent harmonic imaging are shown in US mode (Fig. 1) and in IBS mode (Fig. 2).

Experimental Protocol

To generate a thrombotic coronary occlusion, a copper coil was advanced into the left circumflex coronary artery under fluoroscopic guidance. Coronary occlusion (detected as zero blood flow by the flowmeter) occurred 32 minutes (range, 9–68 minutes) after the insertion of the coil. At 1.5 hours after coronary occlusion, streptokinase was infused intravenously (400,000 IU during 90 minutes). Coronary recanalization (as detected by the restoration of coronary blood flow) occurred, if present, 22
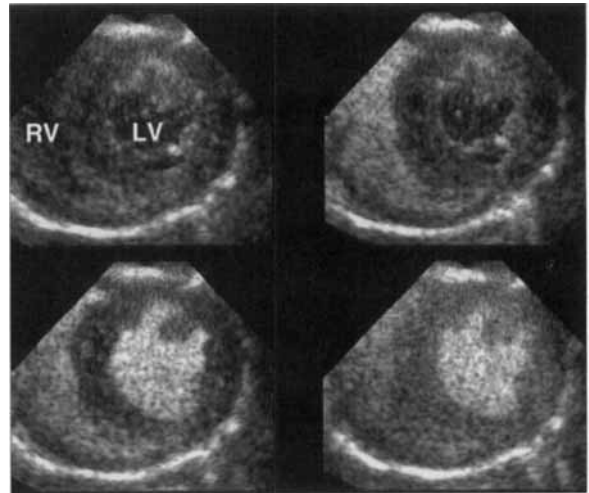

Figure 1. *Two-dimensional echocardiographic images collected by intermittent harmonic imaging in ultrasound mode and corresponding to baseline conditions (left top), right ventricular (RV) cavity enhancement (right top), left ventricular (LV) cavity enhancement (left bottom) and myocardial contrast enhancement (right bottom). RV = right ventricle; LV = left ventricle.*

minutes later (range, 11–44 minutes). Under baseline conditions, during coronary occlusion, and after streptokinase infusion, the echo contrast agent was injected intravenously, and radionuclide microspheres were injected into the left atrium. At the end of the experiment, the animals were killed with intravenous potassium chloride, the heart was removed, and Evans Blue was injected into the left circumflex coronary artery to visualize the myocardium perfused by this vessel.

Data Analysis

Conventional echocardiographic images were digitized off line with a PC-based system on a 256×256 pixel matrix with eight bits of intensity range. For each echo contrast injection, end-diastolic images were digitized, including at least three beats before contrast appearance in the LV cavity and lasting up to its apparent reduction. To evaluate the effects of gain setting on MCE, two regions of interest were drawn in the digitized images corresponding to baseline conditions (i.e., before coronary occlusion). One
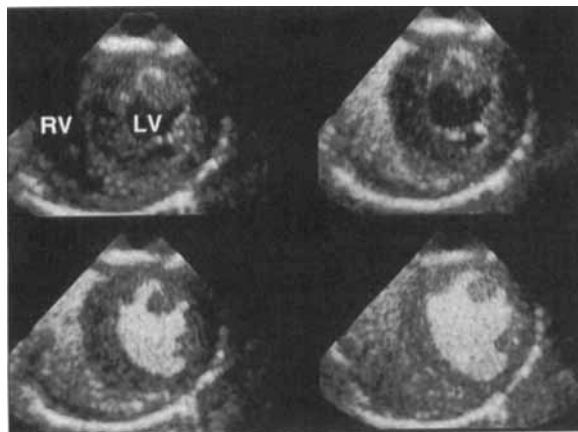
Figure 2. *Echocardiographic images collected by intermittent harmonic imaging in integrated backscattered mode and corresponding to baseline conditions, right and left ventricular cavity enhancement, and myocardial contrast effect (panels and abbreviations as in Fig. 1).*

region corresponded to LV cavity and the other corresponded to the myocardium of anterior wall. These regions were as large as possible without including the specular reflectors of endocardial and epicardial edges or regions of apparent artifacts. The video intensity inside these regions was measured to build LV cavity and myocardial time-intensity curves.

Echocardiographic images recorded with intermittent harmonic imaging were analyzed with a program built-in the scanner (Acoustic Densitometry, Hewlett Packard). In each of the triggered images corresponding to baseline conditions, two regions of interest were located as with conventional imaging: in the LV cavity and in the myocardium of anterior wall. The regions of interest were as large as possible and elliptical and did not include the specular reflectors of endocardial and epicardial edges. Signal intensity inside the regions of interest was measured to build LV cavity and myocardial time-intensity curves, as shown in Figure 3. The curves collected with both conventional and harmonic imaging were analyzed to obtain a background and a peak value.

In the images recorded during coronary occlusion and after streptokinase, both with conventional and harmonic imaging, the area of underperfused myocardium was planimetered

by two independent observers and twice by the same observer and was expressed as percentage of total LV wall area.

Microsphere Analysis

The microspheres had a mean diameter of 15 μm and were labeled with ^{153}Gd, ^{113}Sn, ^{103}Ru, ^{56}Co, ^{51}Cr, and ^{46}Sc (DuPont, New England Nuclear). After staining, the heart was fixed in 10% formalin, and the myocardium was cut into five or six transverse slices. The slice considered to correspond to the cross-sectional echocardiographic view was divided into eight wedges and cut into an inner, a middle, and a outer layer. The sample weights and counts of each piece were transferred to a personal computer. Blood flow per gram of myocardium was obtained according to the formula $Ft = rt \times Fs/rs$, where Ft is flow in each piece (ml/min/g), rt is the radioactivity of each piece (counts/min/g), Fs is arterial reference flow (ml/min), and rs is its radioactivity (counts/min).[33] A piece of myocardium was arbitrarily considered to be underperfused if calculated blood flow was < 50% of flow in the contralateral normal region. Finally, the extent of perfusion defects by microspheres was defined as percentage of the weight of underperfused myocardium divided by the weight of the entire slice.

Statistical Analysis

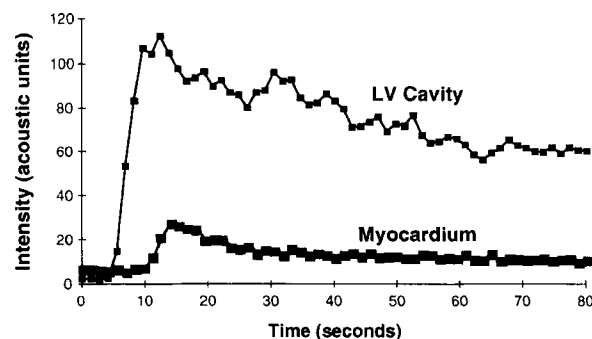The significance of the changes in echocardiographic data before and after contrast adminis-

Figure 3. *Time-intensity curves corresponding to left ventricular (LV) cavity and myocardial contrast enhancement after intravenous contrast injection.*

tration was evaluated by Student's *t*-test for paired data. The same test was used to compare the data obtained in each experiment with intermittent and continuous imaging. The ability to detect myocardial perfusion abnormalities by contrast echocardiography was expressed as sensitivity and specificity. The relationship between the extent of underperfused myocardium by contrast echocardiography and that by radionuclide labeled microspheres was evaluated by least-squares linear regression analysis. P values < 0.05 were considered statistically significant.

Results

A total of 106 injections of echo contrast agent were performed in the study: 34 injections under baseline conditions, 35 during coronary occlusion, and 37 after streptokinase. Conventional imaging was used with 57 injections, and harmonic imaging was used with 49 injections. Each dog was given a mean of 13 injections (range, 9–20 injections).

Acute Hemodynamic Effects

The injection of the contrast agent did not acutely modify heart rate, aortic pressure, pulmonary arterial pressure, or cardiac output (Table I).

Intermittent Versus Continuous Imaging

MCE was higher with intermittent than with continuous imaging. As shown in Figure 4, the increment in myocardial intensity obtained with intermittent imaging was superior to that of continuous imaging using both fundamental and harmonic imaging, both in US and in IBS mode. Conversely, contrast enhancement inside LV cavity was similar with intermittent and continuous imaging (Fig. 5), likely due to signal saturation.

Ultrasound Versus Integrated Backscatter Imaging

Myocardial intensity significantly increased using both US and IBS imaging (Table II). Because these two imaging modalities have different scales—linearized (in acoustic units) for US imaging and logarithmic (in dB) for IBS imaging—it is difficult to establish which imaging modality produced the most intense myocardial contrast effect. Both the imaging modes produced a similar contrast effect at a subjective evaluation.

Perfusion Defects by Contrast Echo Versus Electromagnetic Flowmeter

Figure 6 illustrates myocardial contrast echo images recorded during coronary artery occlusion and reperfusion. Of 41 injections performed during severe coronary underperfusion (arbitrarily defined as electromagnetic coronary flow $\leq$ 20% of baseline value, either before or after streptokinase), myocardial perfusion defects were detected by contrast echocardiography in 39 injections. Of 65 injections

TABLE I

Acute Hemodynamic Effects of the Echo Contrast
Agent NC100100, Intravenously Injected

	Baseline	Contrast	P
Heart rate (beats/min)	127 ± 30	127 ± 31	0.66
Systolic aortic pressure (mmHg)	108 ± 20	111 ± 19	0.03*
Diastolic aortic pressure (mmHg)	85 ± 17	87 ± 17	0.09
Systolic pulmonary pressure (mmHg)	20 ± 5	20 ± 5	0.54
Diastolic pulmonary pressure (mmHg)	8 ± 3	8 ± 3	0.81
Mean left atrial pressure (mmHg)	4 ± 2	4 ± 2	0.77
Cardiac output (l/min)	1.9 ± 0.5	2.1 ± 0.8	0.26
Diastolic coronary flow (ml/min)	15 ± 13	15 ± 13	0.88
Systolic coronary flow (ml/min)	6 ± 8	5 ± 8	0.51

Means ± SD values are presented. * = The very small increase in systolic aortic pressure is statistically significant, but there is a lack of biological relevance.
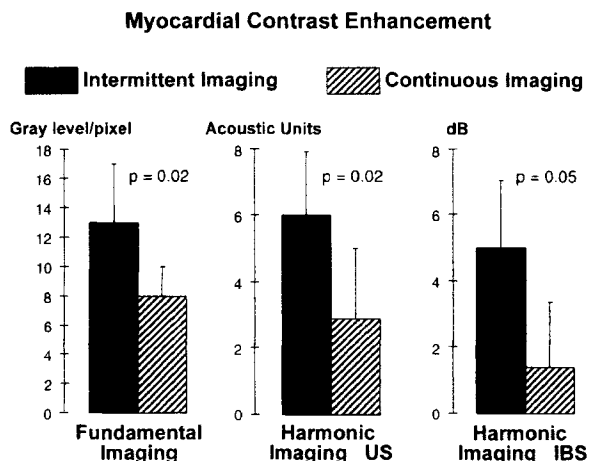
Myocardial Contrast Enhancement

Figure 4. *Increment in myocardial intensity after contrast administration with intermittent imaging and with continuous imaging in the injections performed under baseline conditions. Data were collected by fundamental and harmonic imaging, both in ultrasound (US) and in integrated backscatter (IBS) mode.*

performed with a circumflex blood flow of > 20% of baseline (before or after streptokinase), myocardial perfusion defects were present by contrast echo in 7 injections. Six of these injections were the first ones performed immediately after coronary recanalization. Thus, taking into account the coronary flowmeter as the gold standard, the sensitivity of MCE in detecting severe coronary underperfusion was equal to 95% and the specificity was equal to 89%.

Perfusion Defects by Contrast Echo Versus Microspheres

Of 44 injections corresponding to myocardial perfusion defects by microspheres, 42 also showed perfusion defects by contrast echo. Of 28 injections corresponding to a homogeneous myocardial perfusion by microspheres, 2 showed perfusion defects by contrast echo. Thus, considering radiolabeled microspheres as the gold standard, the sensitivity of MCE in detecting perfusion defects was equal to 93% and the specificity was equal to 95%. The mean difference between repeated measurements of perfusion defects by echo contrast was equal to 5% and 7% of LV cross-sectional area for in-

traobserver and interobserver variability, respectively.

Detection of Myocardial Reperfusion

Of the 37 injections of contrast echo performed after streptokinase, 28 corresponded to an adequate myocardial reperfusion and 9 corresponded to perfusion defects by microspheres. Myocardial contrast echo showed perfusion defects in 2 injections performed during adequate reperfusion and in 8 of the 9 performed after unsuccessful thrombolysis. Accordingly, the sensitivity of contrast echo in detecting myocardial reperfusion was equal to 89% and the specificity was equal to 93%.

Extent of Perfusion Defects by Contrast Echo and Microspheres

The extent of myocardial perfusion defects by contrast echocardiography corresponded to 30% ± 11% of LV myocardium in the cross-sectional view and was not statically different from the extent of perfusion defects by microspheres (27% ± 11% of LV myocardium, P = 0.18). Considering all the injections performed during coronary occlusion and reperfusion, the extent of perfusion defects by contrast echo showed a good correlation with that by microspheres (correlation coefficient r = 0.71).
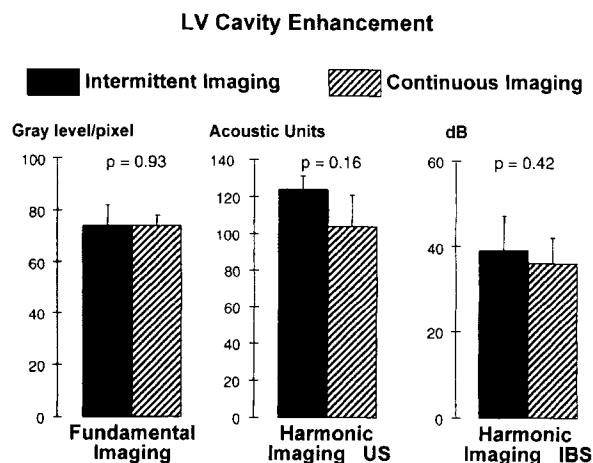
LV Cavity Enhancement

Figure 5. *Peak contrast enhancement in the LV cavity obtained with intermittent and continuous imaging. (Abbreviations as in Fig. 4.)*

TABLE II

Changes in Myocardial and Left Ventricular Cavity Intensity After
Intravenous Contrast Administration Obtained With Ultrasound
and With Integrated Backscatter Harmonic Imaging

	Baseline	Contrast	P
Myocardium US HI (acoustic unit)	8 ± 2	12 ± 6	< 0.01
Myocardium IBS HI (dB)	20 ± 3	23 ± 2	< 0.01
LV cavity US HI (acoustic unit)	4 ± 2	114 ± 16	< 0.01
LV cavity IBS HI (dB)	11 ± 2	36 ± 6	< 0.01

US = ultrasound; HI = harmonic imaging; IBS = integrated backscatter; dB =
decibel; LV = left ventricular.

Fundamental Versus Harmonic Imaging in Detecting Perfusion Abnormalities

The correlation between the extent of myocardial perfusion defects by MCE and microspheres was closer for the 31 injections performed with harmonic imaging (r = 0.82, SEE = 10.27) than for the 38 injections performed with fundamental imaging (r = 0.53, SEE = 12.85).

Discussion

This study shows that the echo contrast agent NC100100 is able to enhance the signal from both LV cavity and the myocardium after an intravenous administration. As already demonstrated with other contrast agents, the myocardial contrast effect caused by NC100100 was higher with intermittent than with continuous imaging. It is already known that diagnostic ultrasound pressures[29,30,34] destroy contrast microbubbles. Thus, delivering fewer ultrasound pulses can destroy fewer microbubbles, resulting in improved contrast effect with intermittent compared with continuous imaging. On the other hand, intermittent imaging prevents the analysis of ventricular wall motion, which is a well-validated tool in the diagnosis of ischemic heart disease. If an adequate myocardial enhancement could be obtained with continuous imaging, this "conventional" approach might be preferable in the clinical arena. At variance with the myocardium, contrast enhancement in the LV cavity did not improve with intermittent imaging, likely due to signal saturation with fundamental imaging.

In this study, the ability of IBS imaging to improve the detection of contrast microbubbles in the myocardium also was evaluated. So far, IBS has been mainly used to provide information on myocardial tissue alterations,[31,32] but it also has theoretic advantages in MCE. Integrated backscatter and ultrasound imaging, however, can hardly be compared because they have different scales. A visual analysis of contrast echo images did not show a superiority of any of these two technique as to MCE, suggesting that conventional imaging can be well used for myocardial perfusion studies in the clinical arena. These data are in agreement with those of Ismail et al.,[35] who did not find any significant difference in the derivation of parameters from time-intensity plots during MCE using IBS or conventional echocardiography, and they also are agreement with a recent report by Aeschbacher et al.[36]
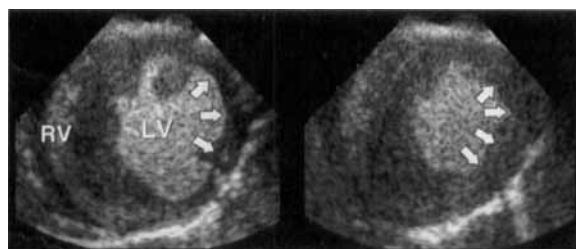

Figure 6. *Myocardial contrast echo images recorded during coronary occlusion (left) and after reperfusion (right). A myocardial perfusion defect can be appreciated during occlusion (arrows), which completely disappeared after streptokinase infusion.*

The MCE obtained with the venous administration of NC100100 and intermittent harmonic imaging is in the range of visible changes in signal intensity. In this study, contrast echo images allowed accurate detection of both myocardial perfusion defects during acute coronary occlusion and myocardial reperfusion after thrombolysis. In the patients with acute myocardial infarction, this observation has relevant implications because early myocardial reperfusion is associated with lower short- and long-term mortality rates.[37] At present, the identification of myocardial reperfusion or failed reperfusion after thrombolysis remains a clinical challenge and is based on clinical variables such as resolution of chest pain, resolution of ST-segment elevation, or occurrence of ventricular arrhythmias.[38] In this situation, MCE might be a reliable noninvasive tool to identify myocardial reperfusion or failed reperfusion after systemic thrombolysis. Finally, the extent of myocardial perfusion defect by contrast echocardiography provided assessment of myocardial perfusion abnormalities very similar to those obtained by radiolabeled microspheres, mainly if harmonic imaging is used.

The study is affected by some limitations. An optimal image quality, which favors the detection of a mild contrast effect, can easily be obtained in an open-chest animal preparation. A good-quality image also can be obtained in humans with transesophageal echocardiography. However, the common clinical scenario for MCE likely is the echo laboratory or the coronary care unit, where the image quality by transthoracic echo often is suboptimal and where new contrast agents and technologies must be validated. Furthermore, the clinical situation is more complex than the experimental setting because coronary occlusions usually last > 2 hours in patients with acute infarction. Finally, conventional and harmonic imaging methods were not performed in the same animal and in a random order, thus preventing a direct comparison of the two techniques.

In conclusion, this study shows that the intravenous injection of the echo contrast agent NC100100 generates an MCE that is measurable and visible and that is favored by the use of second harmonic intermittent imaging. This enhancement allows identification of myocardial perfusion abnormalities in acute infarction and might be of remarkable clinical impact in the handling of patients with acute ischemic syndromes.

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