Assessment of Left Ventricular Area at Risk by Myocardial Contrast Two-Dimensional Echocardiography:

An Evaluation of a New Animal Model

STÉPHANE LAFITTE, M.D.,* PATRICK LAFFORT, M.D.,* DANIELE ROVAI, M.D.,† PHILIPPE ALZIEU,* SIMONE BONORON, PH.D.,* PIERRE BESSE, M.D.,* and RAYMOND ROUDAUT, M.D.*

*INSERM 441, Atherosclerose, Pessac, France; and †Instituto di Physiologia, Pisa, Italy

Background: *New echocardiographic contrast agents are commonly tested in the dog model. However, this species has a number of drawbacks, including difficulties in experimentation, cost, and ethical considerations. The rabbit has a number of advantages due to its relative simple coronary circulation. The present study was designed to evaluate the rabbit model for determination of areas of risk (ARs) by contrast echocardiography.* Methods: *Eight rabbits were intubated and mechanically ventilated after occlusion of the left coronary artery with a ligature. The transducer (operating at 7.5 MHz) was positioned on the right ventricle through a right thoracotomy. The images were obtained after intra-aortic injection of 1 ml of Albunex, followed by 3 ml of dye (Blue Uniperse) for histological analysis postmortem. The ARs were obtained after circumscription of the various echocardiographic and histological images.* Results: *Excellent echocardiographic images were obtained, largely due to the hemodynamic stability of the rabbit to ischemia. Echocardiographic ventricular areas, absolute AR, and relative AR correlated closely with postmortem data (r = 0.86, 0.94, and 0.94, respectively). The measurements were highly reproducible with low variability.* Conclusions: *The rabbit model shows promise for study by contrast echocardiography of myocardium subjected to ischemia. This method for determination of ARs was validated against postmortem findings. The method also should be of value in the evaluation of reperfusion. (ECHOCARDIOGRAPHY, Volume 15, August 1998)*

contrast echocardiography, Albunex, experimental animals, area at risk

The synthesis of new contrast agents with innovative properties has given a new impetus to the use of experimental contrast echocardiography.[1-5] Because lung crossing is essential after an intravenous injection, significant and analyzable myocardial signal enhancement requires agents of high molecular stability.

The testing of new agents requires a suitable animal model, and the dog has been most widely used, mainly for reasons of size. However, apart from the high cost and the infrastructure necessary for animal management, the canine model is limited by its low tolerance of the ischemic state with a high mortality rate during coronary occlusion.[6] It is not a simple model of myocardial ischemia in view of the complexity of the coronary network, although it is close to that of the human one. Interpretation of the perfusion data and their relationship with heart function remains a matter of discussion.[7,8] Because the canine model also raises medicolegal issues, another species would be preferable.

Address for correspondence and reprints: Stéphane Lafitte, 26 rue Virginia, Res les Buissonnets, 33200 Bordeaux, France. Fax: 33-55-6556886.

The rabbit model has been little used for contrast echocardiographic studies, featuring in fewer than 1% of the studies reported during the past decade. Nevertheless, two recent studies have demonstrated the validity of the rabbit model for investigation of the hemodynamic tolerance of infusions of Albunex® (Molecular Biosystems, San Diego, CA, USA) or FS069.[9,10] The rabbit model is convenient and has the advantages of low cost, resistance to ischemia, and suitability for study of energy metabolism. This model thus might compete with the canine model despite its reduced size.

The present study was designed to evaluate the rabbit model for use in contrast echocardiography. The initial objective was to compare intra-aortic infusion of Albunex with the blue dye histopathological method for localization and measurement of areas at risk (ARs). We also evaluated the digital image subtraction technique, and in a subgroup of animals, we investigated the relationship between size of the AR and the duration of coronary occlusion.

Materials and Methods

Animal Preparation

Eight male adult rabbits weighing 3.7 ± 0.14 kg were used in the experiments. Animals were premedicated with ketamine 0.5 ml/kg body weight IM) and acepromazine (0.5 ml/kg IM) 15 minutes before anesthesia. They were shaved on the thorax and on the anterior cervical area, and electrodes were positioned to obtain an adequate electrocardiographic signal for continuous monitoring. The rabbits were anesthetized with xylasine (0.5 ml/kg body weight IM) and ketamine (0.5 ml/kg IM). Anesthesia was maintained by supplemental injections of xylasine and ketamine. The animals were intubated and ventilated with oxygen-enriched room air (50% air and 50% oxygen) with a positive-pressure veterinary respirator.

The right carotid artery was exposed, and a 5-Fr heparinized catheter was introduced and positioned in the ascending aorta for continuous pressure measurements. The heart was exposed through a left thoracotomy in the fourth intercostal space. The pericardium was opened, the left coronary artery was isolated, and a snare was placed in the middle segment for occlusion of the left anterior descending coronary artery.

The coronary occlusion lasted 30 minutes in four rabbits and 120 minutes in the other four. No reperfusion was carried out, and the animals were killed at the end of each period of coronary occlusion.

Echocardiographic Examination

Two-dimensional (2-D) echocardiograms were obtained using a mechanical wide-angle scanner (Apogee, ATL, Bothell, WA, USA) operating at 7.5 MHz. The transducer was positioned on the right ventricle through a right thoracotomy and was oriented and fixed to obtain a short-axis view of the left ventricle at the midpapillary level. In all studies, a saline gel acted as an acoustic interface between the heart and the transducer. Needles through the heart in the plane of the echocardiographic examination marked the slice of the left ventricle for AR analysis.

Gain controls were adjusted to obtain optimal images at the start of the first experiment and were kept constant throughout the subsequent studies. Image acquisition was synchronized to the electrocardiogram. To maintain myocardial perfusion as constant as possible, respiration was not stopped during contrast injections and data acquisition. Echocardiographic images were recorded on 1.25-cm VHS videotape for subsequent playback and analysis.

Echocardiographic Contrast Agent

Air-filled human serum albumin microspheres produced by sonication (Albunex) were used as the echocardiographic contrast agent. These microspheres have a mean diameter of 4 μm, and 95% have a diameter of < 10 μm. The catheter for injection was placed 5 mm above the aortic valve under echocardiographic control to obtain a homogeneous distribution of the contrast agent in both coronary arteries.

A constant dose of contrast agent (1 ml; doses range, 0.24–0.30 ml/kg) at a concentration of 2×10^8 microspheres/ml was manually

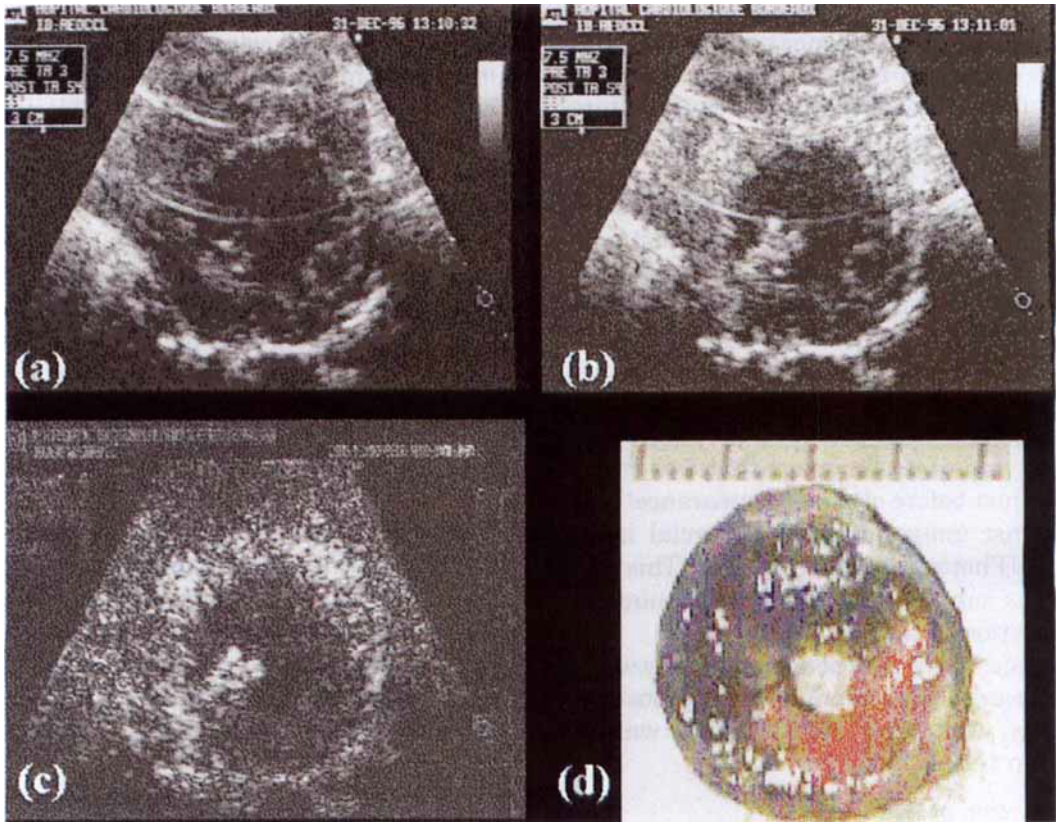

Figure 1. *(a) Myocardial short-axis view before Albunex injection. (b) With contrast injection, the area of risk (AR) is clearly marked off, and the area can be easily measured. (c) The result of subtraction between a and b. (d) Control AR obtained with the blue dye analysis. Contrast echocardiography can correctly assess the size and localization of ARs on our rabbit model.*

injected into the catheter over 2 seconds. The dose and rate of the injection were based on pixel intensity results obtained in preliminary experiments. Echocardiographic images were recorded 30 minutes after coronary occlusion for each rabbit before and after contrast injection (Figs. 1a and 1b). In four rabbits, the size of the AR was evaluated for different durations of occlusion (15, 30, 60, 90, and 120 minutes) with 1 ml of Albunex injection at each stage.

Image Analysis

Echocardiographic images were digitized off-line on a commercially available computer system (Kenitec PC, Mirovideo DC1 system). A 384 × 288-pixel matrix with 256 gray levels was used. Images were digitized at 30 frames per second, including at least five beats before the appearance of myocardial contrast and until disappearance as determined visually (an average of 10 seconds). End-diastolic images were sampled from this sequence of images and stored on a hard disk. For each experiment, the end-diastolic image with the best contrast between the left ventricular cavity and the myocardium was chosen based on the transit time curve.

Each echocardiographic cross section (23 ± 5 mm diameter) was divided into 12 circumferential segments (numbered 1–12) using the posterior right ventriculoseptal junction as a landmark to localize the AR. AR localization was determined by coding each of 12 circumferential segments using qualitative criteria (contrast, no contrast).

For each end-diastolic echocardiographic slice, the total myocardial area (TMA) was

planimetered with a program developed in our laboratory. AR then was measured in a similar way and expressed in both absolute (cm^2) and relative terms (percent of total myocardium) using the following equation: AR (%) for one slice = AR (cm^2)/TMA (cm^2) × 100.

In an attempt to obtain higher correlations, we used the digital subtraction technique described by Armstrong et al,[11] who reported excellent results for the surface measurement of the AR by hydrogen peroxide injection echography. A second measurement of the AR was obtained after digital subtraction. The "only contrast image" was produced by subtracting the latest end-diastolic image that was free of contrast (just before contrast appearance) from the contrast images using commercial image software (Photoshop 1.0) (Fig. 1c). This new image was subjected to a another planimetric determination of the AR.

All images were independently analyzed by two observers. To determine the interobserver variability, the same measurement was repeated ten times.

Determination of AR

At the end of the procedure, 2 ml of Uniperse blue dye was injected into the aortic catheter at the same rate as for contrast injection (0.5 ml/sec). Blue dye was diluted to obtain a similar viscosity to that of Albunex, and the rates of injection were matched to produce identical coronary perfusion. The rabbit then was killed by injection of potassium chloride (5 ml IC), and the heart, stopped in end-diastole, was excised, rinsed with cold water, and cut into 3-ml slices. The slice with needles was photographed and digitized on the computer system (Fig. 1d). A 384 × 288-pixel matrix with 16 million color levels was used, and the measurements of the AR (localization and size) were obtained planimetrically in the same way as for the echocardiographic measurements. The border between normal perfused myocardium and the AR was well defined.

Statistics

All data are expressed as mean ± SD values or as percentages. Linear regression analysis was performed to determine correlation coefficients and residue distribution (Bland and Altman) on the following parameters: (1) TMA measured by echocardiography and blue dye method, (2) absolute and relative AR measured by 2-D contrast echocardiography and by the blue dye method, (3) relative AR measured by simplex contrast imaging and subtraction imaging, and (4) interobserver and intraobserver variabilities. For the latter, the intraclass correlation also was calculated by analysis of variance. For comparison of the localization, a kappa correspondence coefficient was used.

Results

Sensitivity of the Rabbit Model to Ischemia

Ischemic areas were produced in all eight rabbits. Only one had a complication, ventricular fibrillation, which appeared 20 minutes after the occlusion and was stopped by internal cardiac massage. In this animal, injection of blue dye after 10 minutes demonstrated a large AR (63% of TMA). The average AR for all animals was 37% ± 20%.

Hemodynamic Parameters

Aortic blood pressure and heart rate were recorded during each experiment apart from the 2 seconds of Albunex injection. These parameters were not significantly different before and after injection of the contrast agent [peak aortic pressure: before injection, 95 ± 9 mmHg; after injection, 99 ± 7 mmHg; P, not significant (NS); minimum aortic pressure: before injection, 59 ± 7 mmHg; after injection, 63 ± 6 mmHg; P, NS], although peak aortic pressure fell after the coronary occlusion (before occlusion, 95 ± 9 mmHg; after occlusion, 71 ± 12 mmHg; $P < 0.05$).

AR Localization

For each rabbit, 12 sectors obtained by 2-D contrast echocardiography were compared with the same 12 sectors obtained by the blue dye method (Table I). The analysis differed between the two techniques in only six sectors, and the kappa correspondence coefficient (0.86) indicated a good agreement between the two groups.

TABLE I

Comparison of AR Localization

		Contrast Echo		
		Perfusion	Risk Area	Total
Blue dye analysis	Perfusion	56	2	58
	Risk area	4	34	38
	Total	60	36	96

$x = (po - pc)/(1 - pc)$ $x = (0.93 - 0.52)/(1 - 0.52)$

$x = 0.86 (>>0.6)$

pc = global proportion by chance; po = global proportion observed.

AR Size

In these eight animals, 16 short-axis echocardiographic images (eight with subtraction and eight without subtraction) were analyzed for AR.

Without digital subtraction, the TMA ranged from 1.94 to 2.82 cm^2. The nonperfused areas were easily recognized and measured with an excellent contrast between ventricle and myocardium and a less well defined contrast between myocardium and mediastinal tissues. The absolute AR was 0.75 ± 0.36 cm^2 and the relative AR was 33 ± 18%. For the blue dye analysis, the TMA ranged from 1.86 to 2.76 cm^2, with an absolute AR of 0.81 ± 0.39 cm^2 and a relative AR of 37 ± 20%.

The correlation between the values of TMA obtained by the two methods was fair (r = 0.86; y = 0.86x + 0.39; standard error of estimate [SEE] = 0.22) with a homogeneous residue distribution. For AR, the correlations were high for both the absolute (r = 0.94; y = 0.88x + 0.03; SEE = 0.1) and relative (r = 0.94; y = 0.86x + 0.016; SEE = 0.07) values (Fig. 2).

The TMA and the relative and absolute ARs were recalculated from the contrast subtraction images and compared with those obtained with the blue dye technique by regression analysis (Fig. 3). The correlations were comparable to those obtained without subtraction. For the relative AR, the slope was a little less (y = 0.83x + 0.2; SEE = 0.13), but the correlation coefficient was higher (r = 0.97).

There was an excellent correlation (r = 0.99; y = 0.93x + 0.02; SEE = 0.03) between the relative ARs obtained from the echocardiographic images before and after subtraction (Fig. 3). We did not find any significant improvement in correlations or reproducibility after digital processing. The processed images appeared less clear, hindering accurate identification of the endocardial and epicardial outlines.

The lack of myocardial homogeneity after injection of the contrast agent was attributed to destruction of the microbubbles by ultrasound at the frequency used (7.5 MHz). This destruction occurred more in the proximal field than in the distal area because of the acoustic power that varies with the depth.

Relationship Between AR and Duration of Occlusion

For a subgroup of four rabbits, we evaluated the size of the AR by contrast echocardiography after different durations of occlusion (15, 30, 60, 90, and 120 minutes) (Fig. 4). The SD values of the 20 analyzed areas for the four rabbits ranged from 0.03 to 0.05 cm^2, with a mean of 0.04 cm^2. Analysis of variance did not show any significant difference (relative error, 14%). In this small group of animals, we did not find any relationship between AR and duration of ischemia.

Measurement Variability

We found a high correlation between investigators for the detection and sizing of perfusion defects by the echocardiographic and blue dye techniques (r = 0.95; y = 1.02x – 0.07 and r = 0.96; y = 0.94x + 0.07) (Tables II and III). The intraobserver correlations and error values for both methods also were good.

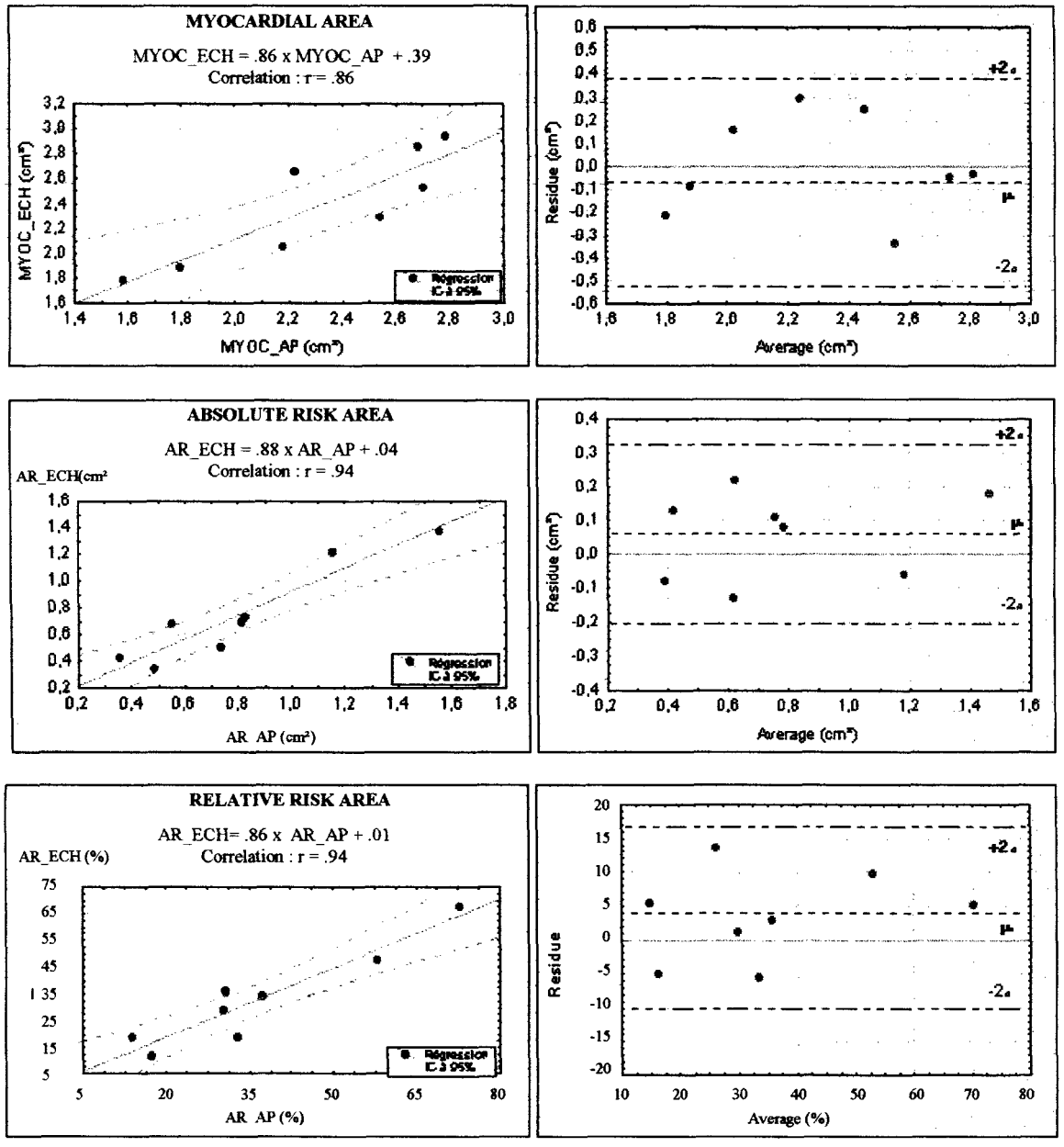

Figure 2. *Correlations between the echocardiographic images (ECHO) and anatomopathology (AP) with blue dye images. (Top) Myocardial areas. (Middle) Areas of risk (ARs) with absolute measures. (Bottom) ARs with relative measures. (Right) Bland and Altman figures of residues. The correlations are very good in the three cases with homogeneous repartition of residues.*

Discussion

Our main objective was to develop a new experimental animal model in which to assess the use of contrast agents for echocardiography. Contrast agents are being developed to assess myocardial perfusion after intravenous injection. These new contrast media are first tested in animals to evaluate their efficacy and possible toxicity. For echocardiographic contrast studies, the animal model must fulfill the criteria imposed by ultrasonic technology: the

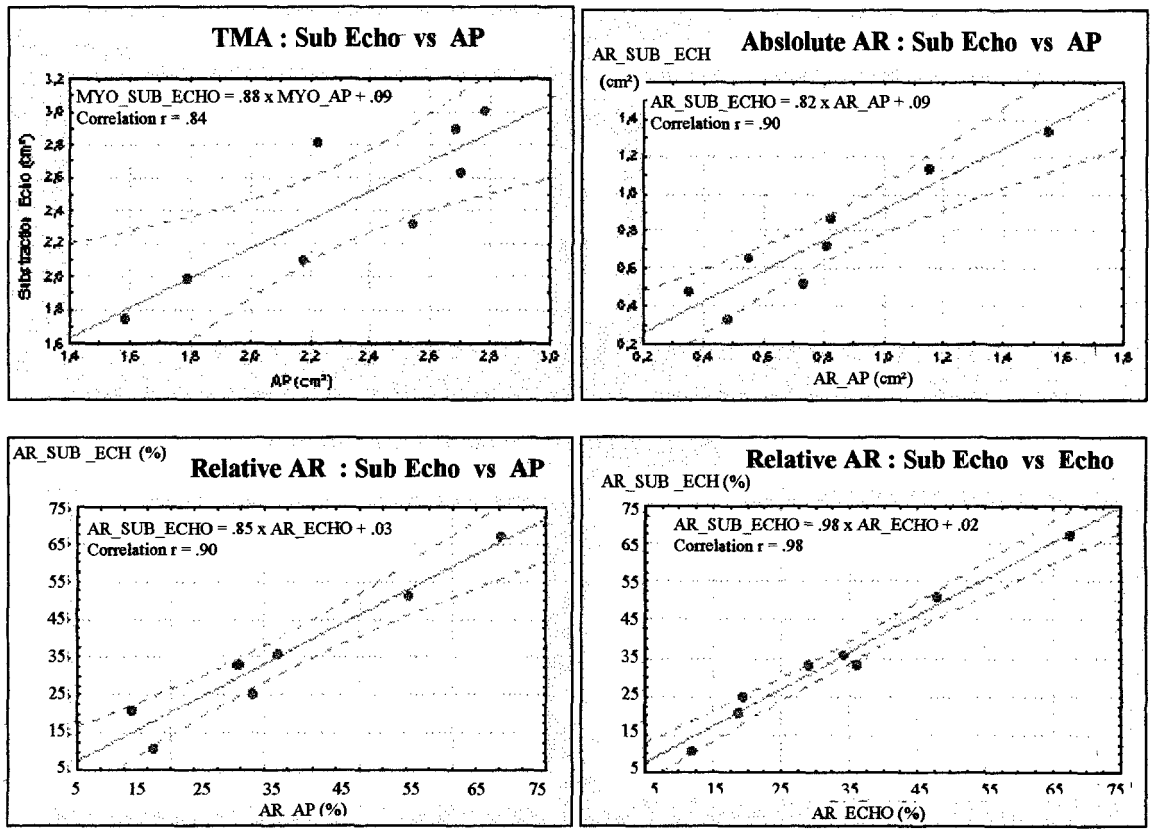

Figure 3. *Correlations between echocardiographic subtraction images and blue dye analysis for total myocardial area (TMA; top left) and relative area of risk (AR; bottom left). (Right) Correlation between echocardiographic images with and without subtraction for TMA (top) and relative AR (bottom).*

spatial and temporal resolutions that are dependent on the probe quality and enable echocardiographic examination of large, slow hearts. For these reasons, the dog is widely used and has become the reference model.

During the past 5 years, considerable advances have been made in probe resolution and contrast enhancement technology (transient response imaging,[12] harmonic imaging,[13] radiofrequency analysis,[14] and Doppler energy[15]) and new contrast agents (EchoGen, Sonus Pharmaceuticals, Bothell, WA, USA). This has enabled a considerable reduction in analyzable tissue volume without loss of information, and some research teams are using the rabbit model for echocardiographic studies.[9,16-19] For contrast echocardiography, Mor-Avi et al[10] demonstrated the high sensitivity of the rabbit model for assessing the hemodynamic effects of intravenous injections of Albunex and FS069.

To test the feasibility of myocardial contrast echocardiography (MCE) in the rabbit, we determined TMA and AR in view of their importance in the study of myocardial ischemia.

Risk Area Measurement

Regional abnormalities in myocardial perfusion have been observed by MCE in dogs after coronary occlusion.[6,20-22] The extent of the abnormalities has been found to be correlated with that observed by autoradiography and dual radioisotope techniques. Determination of the AR using contrast agents is fundamental to the validation of a new experimental model and essential for evaluation of echocardiographic measurements on a smaller heart volume. We adapted the procedures developed in the dog to the rabbit model. Good agreement was found for localization, and the correlations

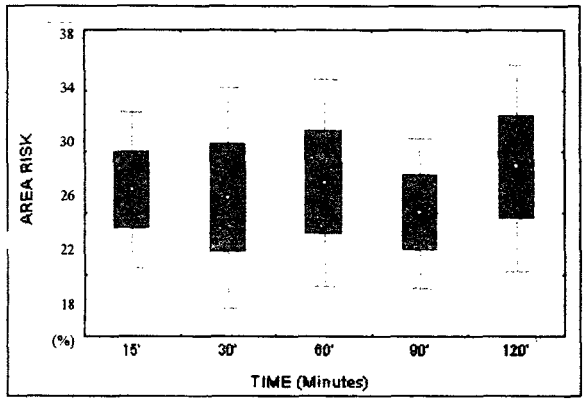

Figure 4. *Relationship between relative area of risk (AR) and duration of occlusion.*

we obtained were comparable to those obtained by Kaul et al[6] with a Renografin mixture and Villanueva et al[21] with sonicated Albumin microbubbles.

In our study, the echocardiographic technique underestimated the TMA but not the AR. This could be explained by the postmortem diastolic state achieved with potassium chloride: in the next moment after excision and despite cold water immersion, we observed a little retraction of myocardial tissue that did not correspond to the in vivo end-diastolic state.

The MCE was homogeneous in the healthy area, and the nonperfused area was well defined, leading to good reproducibility in the measurements.

The intracoronary dye injection technique, which is widely used for postmortem determination of AR, was used as a reference. This method was thought to be more accurate than autoradiographic measurements.

We checked the reproducibility of the measurements and found the same low variability as that obtained in the canine model. This indicated that the size of the rabbit myocardium is not a limiting factor and that myocardial perfusion could be assessed reliably in this model by MCE. We also showed that AR could be assessed by contrast echocardiography without digital image processing in this model.

Relationship Between AR and Duration of Occlusion

We failed to observe any relationship between size of AR and duration of the occlusion. The constancy of the nonperfused areas in ischemia is a matter of debate, but our results are in agreement with those of Kaul et al[6] and West et al,[23] who analyzed AR over longer periods: 6 and 48 hours respectively. In contrast, Kemper et al[24] found a relationship between AR and duration of occlusion after 2 hours. They observed contrast enhancement in epicardial areas in the dogs in their study. This probably was a result of a collateral circulation induced by the ischemia. We were particularly interested in alterations in AR during the first 120 minutes of the ischemia. The initial necrosis did not appear to be increased by prolongation of the occlusion in our rabbit model.

Our results were attributed to the almost complete lack of coronary collateral network in the rabbit during ischemia, unlike that observed in the dog. This also has been described by Cohen et al[25] during controlled coronary occlusion in the conscious rabbit. They also did not observe any proliferation of the coronary network after occlusion repetition.

The absence of a collateral coronary network (either initially or induced) also could explain

TABLE II

Interobserver Variability Analysis

	Echo	Blue Dye Analysis
Interobserver relative error	6.8%	6%
Interobserver intraclass correlation coefficient	0.98	0.99
Interobserver correlation	$y = 1.02x - 0.07$ $r = 0.95$	$y = 0.94x + 0.07$ $r = 0.96$

TABLE III

Intraobserver Variability Analysis

	Echo	Blue Dye Analysis
Intraobserver relative error	5.1%	6.1%
Intraobserver intraclass correlation coefficient	0.96	0.98
Intraobserver correlation	$y = 0.96x + 0.1$ $r = 0.96$	$y = 0.92x - 0.04$ $r = 0.97$

the rapid onset of necrosis in the AR in the rabbit model.

Rabbit Myocardium and Ischemia

Contrast echocardiography has its main application in the study of myocardial perfusion in ischemic heart disease. Experimental studies of myocardial ischemia have used a variety of animal models, although the dog is most widely used. In view of its size, the canine model is convenient, and myocardial ischemia is readily induced by coronary occlusion. However, the tolerance of this model to ischemia appears to be rather variable. Weisel[26] reported that the production of suitable myocardial stunning required a longer duration of coronary occlusion in the dog than in other animals (sheep, rat, pig, and rabbit). This is attributed to the development of a coronary collateral circulation in this species. In contrast to the dog, myocardial stunning and necrosis are easier to obtain in the rabbit, although only by surgical intervention.

With respect to acute ischemic tolerance, the dog often has arrhythmias such as ventricular tachycardia or ventricular fibrillation, and the accidental mortality rate is correspondingly high. Kaul et al[6] reported an overall mortality rate of > 30% in a study using 40 dogs. We occluded coronary vessels in eight rabbits for 30 minutes, and only one presented a reversible ventricular fibrillation. It is noteworthy that this animal had an AR exceeding 60%. The mean AR of the seven unscathed animals was 29% ± 10%. This excellent tolerance also was reported by Cohen et al,[25] who repeated coronary occlusions on seven rabbits with the use of a pneumatic balloon. Only one of the rabbits presented a ventricular fibrillation, which was irreversible. The rabbit model thus appears to offer a good tolerance to acute ischemia.

Study Limitations

The main limitation of the rabbit model in contrast echocardiography studies derives from the lack of extrapolatibility to adult humans. The high frequency transducers (7.5 MHz), which are essential for analysis of small volumes such as the rabbit heart, are not used in human adults; they are most frequently used for investigations on children, who do not require the contrast echocardiography modality. Extrapolation of the results obtained with high frequency transducers to the 2- to 2.5-MHz probe also is not possible because of the microbubble reaction, which differs with the probe frequency. The rabbit model could be used in the early development of contrast agents to assess the hemodynamic tolerance, myocardial contrast, and so on. Despite the lack of the second harmonic modality with high frequency transducers, the rabbit model may have application in transient response imaging, radiofrequency analysis, or Doppler energy mode.

Conclusion

Our results indicate the interest in the use of the rabbit model for the evaluation of echocardiographic contrast agents. The AR as assessed by echocardiographic measurements after intra-aortic injection of Albunex agreed

well with that obtained by the blue dye method postmortem.

The rabbit model is an interesting alternative for the development of new echocardiographic contrast agents due to its convenience, low cost, and other characteristics comparable to those of other commonly used experimental animals.

References

1. Porter TR, Kricsfeld A, Deligonul U, et al: Detection of regional perfusion abnormalities during adenosine stress echocardiography with intravenous perfluorocarbon-exposed sonicated dextrose albumin. *Am Heart J* 1996;132: 41-47.
2. Leischik R, Beller KD, Erbel R: Comparison of a new intravenous echo contrast agent (BY 963) with Albunex for opacification of left ventricular cavity. *Basic Res Cardiol* 1996;91:101-109.
3. Correas JM, Quay SD: EchoGen emulsion: A new ultrasound contrast agent based on phase shift colloids. *Clin Radiol* 1996;51:11-14. Abstract.
4. Grayburn PA, Erickson JM, Escobar J, et al: Peripheral intravenous myocardial contrast echocardiography using a 2% dodecafluoropentane emulsion: Identification of myocardial risk area and infarct size in the canine model of ischemia. *J Am Coll Cardiol* 1995;26:1340-1347.
5. Dittrich HC, Bales GL, Kuvelas T, et al: Myocardial contrast echocardiography in experimental coronary artery occlusion with a new intravenously administered contrast agent. *J Am Soc Echocardiogr* 1995;8:465-474.
6. Kaul S, Glasheen W, Ruddy T, et al: The importance of defining left ventricular area at risk in vivo during acute myocardial infarction: An experiment evaluation with myocardial contrast two-dimensional echocardiography: *Circulation* 1987;75:1249-1260.
7. Rovai D, Ghelardini G, Lombardi M, et al: Myocardial washout of sonicated iopamidol does not reflect the transmural distribution of coronary blood flow. *Eur Heart J* 1993;14:1072-1078.
8. Kates MA, Meza MF, Barbee RW, et al: Potential clinical implications of abnormal myocardial perfusion patterns immediately after reperfusion in a canine model: A myocardial contrast echocardiography study. *Am Heart J* 1996;132:303-313.
9. Mor-Avi V, Shroff SG, Robinson KA, et al: Effects of left ventricular pressure on sonicated albumin microbubbles: Evaluation using an isolated rabbit heart model. *J Am Soc Echocardiogr* 1996;9:156-165.
10. Mor-Avi V, Shroff SG, Robinson KA, et al: Echocardiographic contrast agents and left ventricular contractility: Evaluation using an isolated rabbit heart model. *J Am Soc Echocardiogr* 1996;9:452-461.
11. Armstrong W, West S, Dillon J, et al: Assessment of location and size of myocardial infarction with contrast enhanced echocardiography. II. Application of digital imaging techniques. *J Am Coll Cardiol* 1984;4:141-148.
12. Porter TR, Xie F: Transient myocardial contrast after initial exposure to diagnostic ultrasound pressures with minute doses of intravenously injected microbubbles: Demonstration and potential mechanisms. *Circulation* 1995; 92:2391-2395.
13. Villanueva FS, Kaul S: Assessment of myocardial perfusion in coronary artery disease using myocardial contrast echocardiography. *Coron Artery Dis* 1995;6:18-28.
14. Angermann CE, Kruger TM, Junge R, et al: Intravenous Albunex during transesophageal echocardiography: Quantitative assessment by videodensitometry and integrated backscatter analysis from unprocessed radiofrequency signals. *J Am Soc Echocardiogr* 1995;8:839-853.
15. Von Bibra H, Horcher J, Erhardt W, et al: Doppler energy, second harmonic and standard gray scale imaging in myocardial contrast echocardiography: A comparative animal study. *Circulation* 1996;94:2624. Abstract.
16. Ryu KH, Tanaka N, Ross J Jr: Effects of a sinus node inhibitor on the normal and failing rabbit heart. *Basic Res Cardiol* 1996;91:131-139.
17. Pogwizd SM: Nonreentrant mechanisms underlying spontaneous ventricular arrhythmias in a model of nonischemic heart failure in rabbits. *Circulation* 1995;92:1034-1048.
18. Gardin JM, Wong N, Alker K, et al: Acute cocaine administration induces ventricular regional wall motion and ultrastructural abnormalities in an anesthetized rabbit model. *Am Heart J* 1994;128(pt 1):1117-1129.
19. Halperin BD, Adler SW, Mann DE, et al: Mechanical correlates of contraction-excitation feedback during acute ventricular dilatation. *Cardiovasc Res* 1993;27:1084-1087.
20. Kaul S, Pandiam NG, Okada RD, et al: Contrast echocardiography in acute myocardial ischemia. I. In vivo determination of total left ventricular "area at risk." *J Am Coll Cardiol*

1984;4:1272-1282.
21. Villanueva F, Glasheen W, Sklenar J, et al: Assessment of risk area during coronary occlusion and infarct size after reperfusion with myocardial contrast echocardiography using left and right atrial injections of contrast. *Circulation* 1993;88:596-603.
22. Sakamaki T, Tei C, Meerbaum S, et al: Verification of myocardial contrast two-dimensional echocardiographic assessment of perfusion defects in ischemic myocardium. *J Am Coll Cardiol* 1984;3:34-38.
23. West SR, Armstrong WF, Dillon JC, et al: Digital subtraction contrast echocardiography: Superior predictor of infarct size than wall motion analysis. (Abstract) *J Am Coll Cardiol* 1985;5:475.
24. Kemper AJ, Force T, Perkins L, et al: In vivo prediction of the transmural acute myocardial infarction using contrast echocardiography. *J Am Coll Cardiol* 1986;8:143–149.
25. Cohen MV, Yang XM, Liu Y, et al: A new animal model of controlled coronary artery occlusion in conscious rabbits. *Cardiovasc Res* 1994; 28:61-65.
26. Weisel RD: Models of myocardial stunning. *J Card Surg* 1993;8(suppl 2):287-290.