

Safety and Efficacy of a New Transpulmonary Ultrasound Contrast Agent: Initial Multicenter Clinical Results

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Myocardial contrast echocardiography has been found to be a safe and useful technique for evaluating relative changes in myocardial perfusion and delineating areas at risk. Although earlier contrast agents required direct delivery into the coronary arteries or aortic root, a new echocardiographic contrast agent, sonicated albumin microspheres (Albunex), has been found to cross the pulmonary circulation in experimental models.

To determine the safety and preliminary efficacy of intravenous injections of Albunex in humans, 71 patients at three independent medical institutions underwent two-dimensional echocardiographic examination before, during and after the administration of three intravenous doses of Albunex, ranging from 0.01 to 0.12 ml/kg body weight. All patients provided a complete history and underwent physical and neurologic examination and laboratory and electrocardiographic evaluation before the injections; all evaluations (except for the history) were repeated at 2 h and 3

days after the injections of Albunex. The efficacy of the injections was qualitatively assessed by two independent blinded observers using a grading system of 0 to +3, with 0 indicating an absence of contrast effect and +3 indicating full opacification of the cavities examined.

All injections were well tolerated and no serious side effects were noted in any of the patients. Irrespective of dose group, a cavity opacification $\geq +2$ was seen in the right ventricle in 212 (88%) of 240 injections and in the left ventricle in 151 (63%) of 240 injections as judged by the independent observers. The degree of ventricular cavity opacification appeared to be dose and concentration related. In conclusion, this first multicenter clinical study of Albunex demonstrates that the contrast agent is safe when administered intravenously and achieves significant transpulmonary passage in a majority of patients.

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Contrast echocardiography has evolved as a diagnostic technique since the early report of Gramiak and Shah (1) describing a contrast effect after injection of indocyanine green dye. This contrast technique can be used to detect

valvular regurgitation (2), identify atrial and ventricular cavities (3), assess septal defects and complex congenital heart disease (4) and measure cardiac output (5); more recently, it has been used intraoperatively to evaluate repair of cardiac valves (6) and myocardial perfusion (7). A variety of contrast agents employed include agitated indocyanine green (1), saline or dextrose solution (8), hand-agitated Renografin-76 (meglumine diatrizoate) (9), various sonicated solutions (10), gelatin microspheres (11), albumin-coated bubbles (12), hydrogen peroxide (13) and lipid emulsions (4).

Specifically, contrast echocardiography has been used after intracoronary or intraaortic injections to assess myocardial perfusion defects (9,13), area at risk (14,15), coronary blood flow (16-18) and coronary flow reserve (19,20). However, to accurately assess perfusion, a contrast agent should meet several criteria. The agent should be biologically inert

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(without systemic hemodynamic effects) and should not alter native coronary blood flow (21). The microbubbles that serve as the ultrasound contrast agent should be of equivalent size and distribution and permit unimpeded microvascular flow (22,23). The microbubbles should exhibit physiologic transit time, yet the contrast effect should be sustained long enough to permit adequate imaging over time. Previous contrast agents have not been tested or generally did not meet these criteria. Several reported agents are hyperosmolar and alter coronary blood flow or systemic blood pressure (20,24) and left ventricular contractility (25,26). Hand-agitated solutions and hydrogen peroxide provide intense ultrasound backscatter through the production of large microbubbles (10), but these microbubbles have been shown (22,27) to impede capillary flow, resulting in prolonged transit times. However, previous contrast agents have been observed (11,12,28-31) to pass successfully through the pulmonary circulation in sufficient concentration to opacify the left ventricle after intravenous injection. Thus, it is feasible to pursue the goal of establishing a reproducible transiently ultrasound agent.

Recently (32-34) sonicated albumin microspheres have been evaluated as an echocardiographic contrast agent. In experimental studies (35), sonicated albumin solutions have been shown to not alter hemodynamics, coronary blood flow or left ventricular contractility. A preliminary clinical investigation (34) using a commercially prepared sonicated albumin contrast agent (Albunex) containing stable air-filled microspheres of known sizes and concentration evaluated the safety and efficacy of performing intravenous studies in patients.

This investigation was designed as a multicenter clinical study to assess the safety of using intravenous injections of Albunex in patients and to systematically evaluate the ability of this agent to opacify the left-sided cardiac chambers after passing through the pulmonary circulation. The quality of the contrast effect obtained with different concentrations of the commercially prepared Albunex microspheres was also investigated.

Methods

The study was carried out in three institutions: the University of Chicago, Chicago, Illinois; Baylor College of Medicine, Houston, Texas and the Thoraxcenter, Erasmus University, Rotterdam, The Netherlands. The protocols used at the University of Chicago and Baylor College of Medicine were identical and are collectively referred to as protocol 1. Protocol 1 included mildly hypertensive patients who received serial intravenous injections of increasing volumes of the contrast agent, containing the same concentrations of microspheres. The protocol at the Thoraxcenter (protocol 2) involved healthy (normotensive) patients who received two different concentrations of the

air-filled microspheres. Specific details of the protocols involved in this multicenter study are outlined next. (Unless specifically indicated, protocols 1 and 2 were identical.) The study protocols were approved by the institutional review boards for human studies of the three participating institutions.

Study patients. Protocol 1. Patients who met the following criteria were asked to enroll in the study: 1) age between 18 and 65 years inclusive; 2) male gender or, if female, with no child-bearing potential; and 3) history of mild hypertension (blood pressure <200 mm Hg systolic, 90 to 109 mm Hg diastolic) controlled on a medical regimen (blood pressure \leq 165/85 mm Hg at supine rest). Exclusion of women of child-bearing age conformed with the usual and customary practice of excluding this group from research drug therapy.

Patients were excluded from the study if they had any of the following: 1) previous transient ischemic attacks, cerebral vascular accident, myocardial infarction or congestive heart failure as documented by medical history or current physical examination; 2) the presence of a known right or left heart shunt, cardiac murmur or valvular disease as documented by previous medical examination or diagnostic studies; 3) a history of chronic obstructive pulmonary disease, asthma or other known lung disease as assessed by previous or current medical examination or pulmonary diagnostic studies; 4) a history of blood product allergy; 5) acute or unstable medical condition requiring hospitalization; 6) life-threatening arrhythmia or heart block of greater than first degree as assessed by a previous or current electrocardiogram (ECG); 7) mental incompetence as assessed by history of Alzheimer's disease, mental retardation or organic brain disease documented in the patient's medical history or discovered during the current examination; or 8) failure to provide signed informed consent.

Protocol 2 (Thoraxcenter). Patients in protocol 2 were healthy volunteers (that is, not hypertensive). The inclusion and exclusion criteria were otherwise identical to those in protocol 1.

Basic study design. Protocol 1. The study design consisted of five periods: 1) prestudy, 2) preinjection baseline, 3) testing, 4) 2 h follow-up, and 5) 3 day follow-up. During the prestudy period, within 7 days before injection, the patient's demographic and medical history data were collected. In addition, a physical and neurologic examination and laboratory evaluation including blood chemistry values, prothrombin time, complete blood count and urinalysis was performed during the prestudy period, immediately before injection (preinjection baseline), 2 h after injection and 3 days after injection. The testing period consisted of five intravenous injections: two of 5% serum albumin (control) followed by three of Albunex. All injections were performed manually approximately 5 min apart to enable vital signs to

be measured after each injection and to assure clearance of the remaining contrast effect from the previous injection.

Patients were enrolled in three groups of 10 patients. Each group received a different range of Albunex volumes containing 5×10^8 microspheres/ml. The first group of 10 patients received volumes of 0.01, 0.02 and 0.04 ml/kg, the second 10 patients received volumes of 0.04, 0.06 and 0.08 ml/kg and the third 10 patients received volumes of 0.08, 0.10 and 0.12 ml/kg. All patients received control volumes of 0.5 and 1.0 ml of 5% serum albumin (PPG, Cutter), respectively, which served as a control medium for the Albunex contrast ultrasound agent.

Protocol 2. The basic study design of protocol 2 differed from that of protocol 1 in that during the testing period, subjects received one injection of 1 ml of 5% human serum albumin followed by six injections of Albunex. The six injections consisted of administering three equal volumes (0.01, 0.02 and 0.04 ml/kg) of Albunex solutions that contained two different microsphere concentrations: 5×10^8 microspheres/ml (low concentration, same as in protocol 1) and 8×10^8 microspheres/ml (high concentration). The order of the injections was the same in each study: injection 1 = 1 ml of 5% human albumin; injection 2 = 0.01 ml/kg of Albunex (low concentration); injection 3 = 0.01 ml/kg of Albunex (high concentration); injection 4 = 0.02 ml/kg of Albunex (low concentration); injection 5 = 0.02 ml/kg of Albunex (high concentration); injection 6 = 0.04 ml/kg of Albunex (low concentration); and injection 7 = 0.04 ml/kg of Albunex (high concentration).

Administration procedure. A standard intravenous infusion containing either 5% dextrose in water or 0.45% or 0.9% sodium chloride from the left or right antecubital vein was performed using an 18 gauge (three patients protocol 1 only) or a 20 gauge angiocatheter connected to a three-way stopcock. After obtaining a satisfactory precontrast echocardiographic image in the apical four chamber view, control injections of 5% serum albumin followed by injections of Albunex were performed.

Two-dimensional echocardiographic examination and contrast evaluation. The following commercially available ultrasound systems were used by the investigators: General Electric Pass II, General Electric RT5000, Advanced Technology Laboratories MK 600, Hewlett-Packard 77020AC or Toshiba SSH/160A. All were equipped with either a 2.5, 3.5 or 5 MHz transducer. Volunteers were examined in either the supine or lateral decubitus position. Apical four chamber views were obtained, and gain, reject and intensity settings were optimized at the beginning of each study and were not changed during the study. Imaging was begun just before each injection and continued for 2 to 3 min after each injection to assure clearance of the contrast agent. Images were recorded on 0.5 in. (1.27 cm) VHS videotape and were later analyzed by the independent blinded observers using the described grading system.

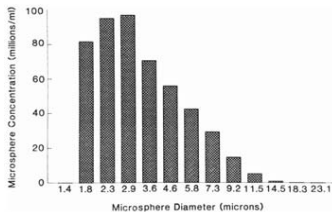


Figure 1. Histogram of a representative sample of the contrast agent demonstrating the size and frequency distribution of the microspheres. The average diameter is 4 μ m.

Safety and efficacy measures. Safety was evaluated by observing the patients for the development of adverse signs and symptoms during the testing and follow-up periods and by laboratory evaluations performed during the testing period. Blood pressure, pulse rate, respiratory rate, temperature and laboratory variables were measured before the testing period and at both 2 h and 3 days after testing. Additional measurements of blood pressure, heart rate and respiratory rate were performed before each injection and after the final injection. Each patient's neurologic status was assessed throughout the testing period. Subjective complaints, changes in mentation and body tone and respiratory problems were recorded as adverse events.

Quality of contrast effect was assessed by observing the magnitude of contrast in the cardiac chambers and was evaluated by opacification grades of 0 (no contrast), +1 (trace), +2 (intermediate) and +3 (full opacification). Two independent blinded observers (P.M.S. and W.F.A.) at institutions not involved in the clinical trials reviewed all injections in a random order. For the efficacy gradings, the blinded observers were not told the agent (control medium versus Albunex), volume or order of injection. The grades assigned by the blinded observers were averaged and reported.

Test materials. Albunex is composed of sonicated human serum albumin in a sterile, nonpyrogenic aqueous solution. The sonication process is performed by the manufacturer (Molecular Biosystems) on the albumin (human) 5% solution, biologic approved by the Food and Drug Administration. In the process, air-filled albumin microspheres are formed, having an average diameter of 4 μ m (Fig. 1).

Statistical analysis. Data were entered into a data base and checked by double entry or manual inspection. Analysis of variance was used to detect changes in vital signs or laboratory data between prestudy, preinjection baseline testing and 2 h and 3 day follow-up periods. Changes were

Table 1. Demographic Data From 71 Patients

	No.	Age (yr)	Height (cm)	Weight (kg)
University of Chicago (protocol 1)				
Group I	10	40.0 ± 10.7	175 ± 11	91.6 ± 14.0
Group II	10	39.5 ± 14.7	180 ± 7	88.1 ± 19.0
Group III	10	49.2 ± 10.9	165 ± 13	78.1 ± 14.0
Baylor College of Medicine (protocol 1)				
Group I	10	48.3 ± 10.7	174 ± 5	94.4 ± 16.5
Group II	10	57.6 ± 7.8	176 ± 7	85.1 ± 17.0
Group III	10	48.5 ± 9.4	175 ± 10	77.0 ± 12.5
Thoraxcenter (protocol 2)				
Group I	11	22.5 ± 2.0	186 ± 6	78.9 ± 8.4

Values are expressed as mean values ± 1 SD. Hypertensive patients were studied under protocol 1 and normotensive patients under protocol 2. Groups I, II and III indicate Alburnex volumes (ml/kg) of 0.01, 0.02 and 0.04 (group I), 0.04, 0.06 and 0.08 (group II) and 0.08, 0.10 and 0.12 (group III).

considered significant at $p \leq 0.05$. Data are expressed as mean values ± 1 SD.

Results

Patient demographic data (Table 1). A total of 60 patients were recruited in protocol 1 and an additional 11 patients in protocol 2. The University of Chicago group I contained only men who were significantly heavier (91.6 ± 14.0 kg) than patients in group III (78.1 ± 14.0 kg, $p < 0.05$), which was composed primarily of women. The three groups did not differ significantly in age or height. The patients in the Baylor College of Medicine group I were significantly younger than patients in group II (48.3 ± 10.7 versus 57.6 ± 7.8 years, $p < 0.05$), but the two groups did not differ in regard to height or weight. Comparing the University of Chicago patients with the Baylor College of Medicine patients, the only significant difference was the age of the patients in group II (39.5 ± 14.7 versus 57.6 ± 7.8 years, $p < 0.01$). The Thoraxcenter patients were younger and taller than the patients at the two other institutions ($p < 0.001$ and < 0.05 , respectively). They were also lighter than the group I patients at the other institutions ($p < 0.02$).

Safety Evaluation

Vital signs and physical and laboratory data (Tables 2 and 3). Significant changes in pulse rate, respiratory rate and temperature were detected by analysis of variance when comparing the 2 h postinjection period with the 3 day postinjection period in patients studied with protocol 1. These data were adjusted for baseline values (prestudy values). However, these adjustments were minor and not thought to be of clinical significance.

A statistically significant change in systolic blood pressure was seen in the protocol 2 patients (Table 3). However, this finding was not considered to be of clinical significance.

Adverse reactions. Adverse reactions were minor and infrequent. These included a feeling of matting on the surface of the eye not accompanied by objective visual deficits in one patient, unusual taste sensation after injections in two patients, lightheadedness on standing at the conclusion of the 2 h study in two patients and erythema near the intravenous catheter site in one patient. No new or significant neurologic deficits or physical findings were noted during the 2 h or 3 day follow-up examinations.

Table 2. Summary of Vital Signs in the 60 Hypertensive Patients in Protocol 1

	No.	Before Study	2 h After Injection	3 Days After Injection	p Value
SBP (mm Hg)	60	137.5 ± 15.0	140.2 ± 11.2	136.8 ± 15.3	NS
DBP (mm Hg)	60	88.0 ± 9.7	87.8 ± 7.5	89.5 ± 9.4	NS
HR (beats/min)	60	71.9 ± 10.3	70.6 ± 7.9*	74.4 ± 10.8*	<0.006
Resp rate (breaths/min)	60	17.1 ± 2.5	17.0 ± 2.8*	15.8 ± 2.3*	<0.005
Temp (°C)	57	36.67 ± 0.32	36.59 ± 0.36*	36.78 ± 0.31*	<0.004

*The analysis of variance (ANOVA) was determined for the 2 h postinjection values as compared with the 3 day postinjection values (adjusted for baseline or prestudy values). Values are expressed as mean values ± 1 SD. DBP = diastolic blood pressure; HR = heart rate; Resp = respiratory; SBP = systolic blood pressure; Temp = temperature.

Table 3. Summary of Vital Signs in the 11 Normotensive Patients in Protocol 2

	Before Study	2 h After Injection	3 Days After Injection	p Value
SBP (mm Hg)	134 ± 6	139 ± 6	128 ± 6	<.001
DBP (mm Hg)	82 ± 5	80 ± 7	79 ± 7	NS
HR (beats/min)	79 ± 8	72 ± 10	79 ± 12	NS
Resp rate (breaths/min)	12 ± 2	12 ± 0.9	11 ± 1.4	NS
Temp (°C)	36 ± 0.3	36.6 ± 0.2	36.7 ± 0.2	NS

Values are expressed as mean values ± 1 SD. p values were determined by analysis of variance (ANOVA). Abbreviations as in Table 2.

No significant differences in the laboratory variables studied during the initial examination and at 2 h and 3 days after the study were detected by analysis of variance for the patients at any of the institutions. No significant ECG changes other than minor nonspecific ST-T wave abnormalities were noted.

Quality of Contrast Effect

Protocol 1 (Table 4). Figure 2 demonstrates sequential opacification of the right and left ventricles after a single injection of 5.9 ml of Albunex. As expected, increasing volumes of Albunex resulted in higher opacification grades

Table 4. Percent of 60 Hypertensive Patients in Protocol 1 Having ≥Grade +2 Cavity Opacification

Patient Group	Volume of Albunex (ml/kg)						
	0.01	0.02	0.04	0.06	0.08	0.10	0.12
University of Chicago							
Right ventricle							
Group I*	22	22	44				
Group II			80	100	100		
Group III					100	100	100
Left ventricle							
Group I*	0	11	11				
Group II			60	80	90		
Group III					90	90	90
Baylor College of Medicine							
Right ventricle							
Group I	90	100	100				
Group II			100	90	100		
Group III				100	100	100	
Left ventricle							
Group I	30	30	60				
Group II			60	60	60		
Group III					90	90	70

*Note that only 9 of the 10 patients in Group I from the University of Chicago were entered into the efficacy analysis because of technical difficulties encountered during the videotape recording of Patient 3. Data are expressed as the mean values determined by two blinded observers. See Table 1 for definition of groups.

in both ventricles. Comparing the group I patients (0.01 to 0.04 ml/kg) at Baylor College of Medicine and University of Chicago, the former patients achieved better opacification in the right (90% to 100% versus 22% to 44%, respectively) and left (30% to 60% versus 0% to 11%, respectively) ventricle. However, comparing the group II patients (0.04 to 0.08 ml/kg), those from the University of Chicago achieved approximately equal opacification of the right ventricle (80% to 100% versus 90% to 100%, respectively) and better opacification of the left ventricle (60% to 90% versus 60%, respectively). Group III patients achieved similar opacification of the right ventricle and 90% achieved +2 opacification of the left ventricle at both institutions; however, group III patients at Baylor College of Medicine achieved 70% of +2 opacification of the left ventricle using the dose of 0.12 ml/kg.

Protocol 2 (Table 5). In Patient 8, contrast was noted to appear almost simultaneously in the right and left atria. An intravenous infusion of hand-agitated saline solution revealed the presence of contrast material in the left ventricle. A presumptive diagnosis of a patent foramen ovale was made and, therefore, the patient was excluded from the efficacy analysis.

There was no difference in right ventricular opacification between the solution containing the lower concentration of microspheres (5×10^8 microspheres/ml) and the solution containing the higher concentration (8×10^8 microspheres/ml). Ninety percent of the subjects achieved at least +2 opacification of the right ventricle, beginning with the smallest volume (0.01 ml/kg). In the left ventricle, however, there was a difference between the two concentrations at a volume of 0.01 ml/kg (50% versus 70%) and 0.04 ml/kg (90% versus 100%). The higher doses (0.04 ml/kg) provided the best left ventricular opacification and produced substantial attenuation of the right ventricular image presumably because of the effects of acoustic shadowing.

Discussion

Safety. This multicenter study represents the first clinical evaluation of the safety and efficacy of a newly developed, commercially prepared transpulmonary ultrasound contrast agent (Albunex). Previous studies (36) on the safety of intravenous injections of hand-agitated agents have reported a low incidence of complications. Similarly, intraarterial and intracoronary injections of both hand-agitated and sonicated contrast agents have generally been found to be safe (7,19,20,35-41). In the present study, no clinically significant laboratory, ECG, physical or neurologic abnormalities were detected after intravenous injections of the contrast agents. A small number of reactions were noted, but were brief and spontaneously resolved. Statistically significant changes in vital signs were detected by analysis of variance; however, these were not recognized to be clinically significant. Based



on these observations, it appears that intravenous administration of this ultrasound contrast agent is safe and that additional clinical experience will be required.

Quality of contrast effect. Previous studies (11,12,28-34) have demonstrated the feasibility of transpulmonary passage of echocardiographic contrast agents. In our study, protocol 1 was designed to determine the volume of Albunex at a low concentration required to opacify the left ventricle after intravenous injections. At both The University of Chicago and Baylor College of Medicine, the use of 0.01 to 0.04 ml/kg was inadequate for left ventricular opacification in the majority of the patients. Increasing the volume to 0.04 to 0.08 ml/kg resulted in +2 opacification in 60% to 90% of patients at The University of Chicago and 60% at Baylor College of Medicine. Interestingly, among patients receiving the low volume dose of Albunex, a higher percent at Baylor College of Medicine than at The University of Chicago achieved +2 opacification of both ventricles. Conversely, for the 0.04 to 0.08 ml/kg dose, patients at The University of Chicago achieved a greater percent of opacification than did those at Baylor College of Medicine.

The apparent differences in contrast opacification observed at the two institutions may be explained by any of the following characteristics: 1. Small sample size. The individ-

Figure 2. Serial frames from a single intravenous injection in a patient whose heart was imaged from an apical four chamber view. A, Baseline image without contrast; B, early image showing contrast in the right ventricle; C, contrast opacification of both left and right ventricles. In these images, the left atrium and left ventricle (LV) are on the left and the right atrium and right ventricle (RV) are on the right.

ual group sample sizes were small ($n = 10$) and the variations between the groups may be due in part to chance.

2. *Subjectivity of opacification grade.* The grading system used (0 to +3 scale) was subjective and relied on the judgment of the independent observers.

3. *Differences between the patient groups.* No attempt was made to ensure that the patient groups were equivalent with respect to age, weight, gender, ethnic origin and so on. Notably the patients in group II from Baylor College of Medicine were significantly older than the group II patients from The University of Chicago. Other differences such as smoking history or minor pulmonary disease could have influenced the degree of opacification.

4. *Differences in pulmonary vascular physiology.* Patients received volumes of Albunex based on their body mass. Presumably, the pulmonary vasculature represents the greatest impediment to passage of contrast material to the left-sided cardiac chambers. Because total pulmonary vascular blood volume is proportional to body surface area rather than mass (42), differences in patients' body habitus may have affected transpulmonary passage of the contrast agent.

5. *Differences in injection technique.* During the studies, patient position (decubitus or supine) was noted to influence the rate of contrast appearance in the right heart chambers after injection. Similarly, differences in the vein placement of the angiocatheter may have influenced the results, as might the rate of Albunex administration (estimated to be 0.5 to 1.0 ml/s).

Table 5. Percent of the 10 Patients in Protocol 2 Having \geq Grade +2 Cavity Opacification

	Volume of Albunex (ml/kg)		
	0.01	0.02	0.04
Right ventricle			
5 \times 10 ⁶ microspheres/ml	90	100	100
8 \times 10 ⁶ microspheres/ml	90	100	100
Left ventricle			
5 \times 10 ⁶ microspheres/ml	50	80	90
8 \times 10 ⁶ microspheres/ml	70	80	100

Data are the mean values determined by two blinded observers.

6. *Contrast agent variability.* During the studies, Albunex solutions from different lot numbers were provided to the investigators by the manufacturer. The investigators did not perform an analysis of contrast agent uniformity. Any variability in microsphere concentration or size could have influenced the degree of cavity opacification.

Despite these differences, +2 or +3 left ventricular opacification was achieved in the majority of patients in groups II and III (0.4 to 1.2 ml/kg). Further studies will be required to determine to what extent variables (volume, body mass, pulmonary disease, injection technique, agent consistency and so on) affect left ventricular opacification in patients.

Protocol 2 differed from protocol 1 in that the subjects were healthy volunteers and Albunex solutions containing two different concentrations of microspheres were administered. At the lower concentration, patients at the Thoraxcenter revealed higher degrees of opacification for the same dose schedule than did group I patients at the other two institutions. This difference may have been due to the characteristics of the Thoraxcenter study group, which included significantly younger, taller and lighter patients than their group I counterparts at The University of Chicago and Baylor College of Medicine, perhaps resulting in better image quality. Improved opacification was observed in protocol 2 with the higher concentrations of microspheres. Our results suggest that both the volume injected and the concentration of microspheres play a major role in the degree of cavity opacification. The higher degree of right ventricular opacification and its frequent attenuation suggest that the volume and concentration needed to study the right ventricle might differ from that needed to study the left ventricle.

The in vitro stability of Albunex has been reported (Molecular Biosystems communication) to be >95% retention at 8 months, which differs significantly from the persistence of ideal gas bubbles such as those of sonicated Renografin-76 (10). Although sonicated Renografin-76 microbubbles disappear rapidly by diffusion, the fate of albumin microspheres appears to result in trapping, primarily in the liver, spleen and reticuloendothelial system, and is dependent on the size of the microspheres (43). The size of Albunex microspheres is sufficiently small that they can pass through the pulmonary circulation to opacify the left ventricle. In the present study, the contrast agent was administered as an intravenous bolus injection. However, by the time the contrast agent reached the right ventricle, it was mixed with blood and no longer passed as a discrete bolus. For a 70 kg subject receiving an Albunex volume of 0.04 ml/kg with a concentration of 8×10^8 microspheres/ml, the ultimate concentration after mixing with the entire blood volume (5 liters) would be approximately 5×10^5 microspheres per ml of blood. This concentration results in a visible attenuation in the ultrasound backscatter intensity and presumably accounts for the image attenuation seen in the right ventricle. In the left

ventricle, however, no visible attenuation of signal was observed during peak opacification. Therefore, the microsphere concentration may be assumed to be significantly lower than that observed in the right ventricle. This loss of contrast effect cannot be explained by trapping in the lungs (de Jong et al., unpublished observations). Therefore, the effects of dilution, cyclic pressure variation or microsphere dissolution may contribute to the loss of contrast effect. Further investigation is required to determine precisely the fate of the microspheres and the physical factors affecting transpulmonary opacification.

Potential clinical applications. Several potential clinical applications for a stabilized ultrasound contrast agent capable of reliable transpulmonary passage exist.

1. *Assessment of left ventricle function.* A recognized limitation of echocardiography in the evaluation of global and regional wall motion derives from endocardial border identification in some patients. Because of the ease of intravenous administration of the contrast agent, the ability of left ventricular contrast medium to enhance edge detection may prove to be useful in detecting wall motion abnormalities in the acute setting and during stress echocardiography (44-46). With opacification provided through the use of this agent, it may be possible to calculate cardiac output or left ventricular ejection fraction using time-intensity variables in a method analogous to radionuclide studies.

2. *Quantitation of valvular regurgitation.* Doppler color flow mapping is currently used to assess mitral and aortic regurgitation. However, the method is velocity rather than volume dependent and is often poorly reproducible (47). It is conceivable that videodensitometric analysis of the regurgitant contrast effect could provide a method of evaluating the degree of valvular regurgitation.

3. *Improved detection of intraventricular flow patterns.* Left ventricular contrast studies may be useful for assessing abnormal intraventricular flow patterns in ischemic heart disease. A recent study by Beppu et al. (46) suggests that in a canine model of experimental myocardial infarction, echocardiographic contrast medium can assist in assessing blood flow pathways that may lead to thrombus formation. Echocardiographic detection of clots or aneurysms may also be significantly improved. Early reports (45,48) have commented on the enhancement of the Doppler signal after contrast appearance.

4. *Perfusion assessment.* Myocardial contrast echocardiography has been shown (7,19,20,37-41) to be a useful clinical technique for defining regional myocardial perfusion. Until now, echocardiographic contrast studies in patients were performed during cardiac catheterization or in the operating room because transpulmonary opacification of the myocardium was not routinely feasible. A preliminary report of a videodensitometric analysis of the patients studied in protocol 2 (Thoraxcenter) by Silverman et al. (49) described the enhancement of myocardial backscatter after intrave-

nous injections of Albunex. The ability of sonicated albumin to cross the pulmonary barrier and the anticipated improvements in digital acquisition of ultrasound signals from units with expanded dynamic range and direct radiofrequency data acquisition (41) may provide the ability to quantitate myocardial perfusion after the intravenous administration of contrast material. If successful, this technique may eventually play a significant role in the diagnosis, evaluation and clinical management of patients with ischemic heart disease.

Conclusions. The present multicenter study is the first report on the clinical use of a commercially prepared ultrasound contrast agent (Albunex). In 71 patients who were either normotensive or mildly hypertensive, no significant adverse physical, neurologic, ECG or laboratory sequelae were detected.

The data also demonstrated the feasibility and reproducibility of left ventricular opacification after intravenous injections of this agent. However, several important questions are raised by the results of this study. What is the optimal dose determination? Should the volume be administered on the basis of body mass or another variable such as body surface area? What are the effects of injection technique and rate on ventricular opacification. What is the fate of the microspheres in the circulation and what factors affect their disappearance? Does a sufficient concentration of microspheres enter the coronary circulation to increase myocardial backscatter enough to assess myocardial perfusion with commercial ultrasound equipment? These questions will require additional experience with this agent and the use of sophisticated quantitative imaging and analysis.

On the basis of the data presented, intravenous injection of Albunex appears to be a safe and effective method for providing left ventricular ultrasound contrast opacification. A standardized ultrasound contrast agent appears to be a major advance in the field of contrast echocardiography.

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