Safety of a New Transpulmonary Echocontrast Agent (Albunex®) in Repeated Echocardiographic Studies in Patients

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Summary

Background and hypothesis: Multiple contrast-enhanced echocardiographic studies are to be expected in patients with cardiac ischemic disease, but the sonication process used to produce the echocontrast agent Albunex® may result in new epitopes that could cause an immunogenic response.

Methods: Repeated exposures to intravenous Albunex over a period of time long enough to allow development of an eventual immune reaction were performed in 12 patients while monitoring for lymphocyte transformation, microsphere specific IgE and IgG antibodies, and systemic, pulmonary artery, capillary wedge, and right atrial pressures, as well as cardiac output, left ventricular fractional shortening, and blood gases.

Results: No significant ³H-thymidine incorporation and thus no specific blastic transformation of the patients' lymphocytes were observed either for high or low Albunex concentrations, corresponding to the expected hepatic and plasma concentrations of microspheres. No formation of microsphere-specific IgE and IgG antibodies was observed after the first or second Albunex exposure. Furthermore, no clinically significant hemodynamic or respiratory adverse reactions were observed in any patient.

Conclusion: These results suggest that repeated exposures to intravenous Albunex induce no adverse effect on the cellular and humoral immune systems and on left and right heart hemodynamics in patients.

Key words: Albunex®, contrast echocardiography, immune system, safety microspheres

Introduction

Recently, myocardial perfusion assessment has been achieved after intracoronary, intracardiac, or intravenous in-

jection of sonicated microbubbles made from 5% human albumin.^{1–3} The utility of contrast-enhanced echocardiography to determine myocardial perfusion defects, area at risk, coronary blood flow, perfusion by collateral flow and cardioplegia, and angioplastic or thrombolytic procedures efficacy,^{4–7} together with the development of contrast stress echocardiography,^{8,9} will lead to a broader and repetitive use of echocontrast agents. Indeed, multiple-contrast echocardiographic studies are to be expected in patients with cardiac ischemia to assess the extent of ischemic heart disease.

Albunex® (Mallinckrodt, Inc., St. Louis, Mo.) is a new transpulmonary echocontrast agent in which the stability, consistent size, and concentration of microbubbles make it useful for improved left ventricular function and myocardial perfusion assessment.^{3,10} The product is made by sonication of a 5% solution of human albumin and consists of air-filled albumin microspheres suspended in the albumin solution.^{11,12} Its safety for successive intravenous or intracoronary injection in most animal species and in humans has been shown after a single exposure.^{13–16} Nevertheless, repeated exposure to Albunex may induce major adverse clinical and hemodynamic events in animals.^{17,18} These adverse events appeared to be the result of sensitization to the human serum albumin fraction, but the antigenicity of the sonicated fraction of Albunex was not addressed.¹⁷

As the sonication process may result in the creation of new epitopes secondary to structural alterations in the albumin molecules, this could cause an immunogenic response in recipients.^{11,12,19,20} We therefore investigated the effect of repeated exposures to this agent on both the cellular and humoral immune systems and left and right heart hemodynamics in instrumented patients with ischemic heart disease. To permit an eventual immunologic response, the second exposure to Albunex was performed after allowing adequate time for the development of specific cellular and humoral immunologic reactions.

Methods

Study Population

The study was carried out in 12 patients with cardiac ischemia (11 men, 1 woman; mean age, weight, and height 53 ± 10 years, 78 ± 10 kg, and 1.72 ± 0.1 meters, respectively) referred for heart catheterization procedures for coronary angiography (4 right coronary artery, 1 circumflex artery, and 7 left descending artery). In all patients, anti-inflammatory

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drugs (acetylsalicylic acid and cortocosteroid) were withdrawn to avoid blunting of an eventual thromboxane-mediated hypertension.¹⁴ Hydroxyzine, an H₁ receptor antagonist, was given to all but three patients for its anxiolytic properties. The protocol was approved by the institutional review boards for human studies and all patients signed an informed consent form before participating in the study.

Study Design and Patients Instrumentation

The study was designed as an open label study in which the patient served as his/her own control. Each patient was exposed twice to Alburnex, 2.7 to 5.7 (4.2 ± 1.5) months apart, to allow time for development of an eventual immunologic reaction. Possible effects of Alburnex on the cellular and the humoral immune systems were examined by assessment of lymphoblastic transformation and of Alburnex-specific IgE and IgG antibodies, performed just before the first and then after the first and the second exposures to Alburnex. Results were compared with similar data obtained in each patient prior to Alburnex exposure. During the second Alburnex exposure, two successive intravenous injections of Alburnex were performed at least 15 min apart to enable vital signs to be measured after each injection. Cardiac output and pulmonary artery, capillary wedge, and right atrial pressures were determined using a #7 French Swan-Ganz catheter placed via a femoral vein. A #7 French introducer sheath was introduced into a femoral artery for systemic blood pressure monitoring and arterial blood sampling, and a #18 G catheter was placed in a brachial vein for injection of Alburnex.

Biochemical Parameters

Arterial blood samples were drawn at 5 min before and at 5 and 10 min after each Alburnex injection to determine arterial blood gases, pH, bicarbonates, and arterial O₂ saturation (Blood Gas System 288, Ciba-Corning, Oberlin, Ohio).

Hemodynamic and Echocardiographic Measurements

All pressures were continuously monitored using a fluid-filled recording system connected to a Honeywell VR-12 multichannel recorder. Representative tracings were recorded on a strip-chart recorder and values were reported at baseline (-5 and 0 min), at 1, 3, 5, 10, and 15 min after the first Alburnex injection, and at 1, 3, 5, and 10 min after the second Alburnex injection. Cardiac output was determined by thermodilution (Cardiac Output Computer COM-1, Baxter Healthcare Corp., Edwards LIS Div., Irvine, Calif.) and was calculated as the mean of three to five measurements. Ten ml of cold saline ($0-3^{\circ}\text{C}$) was injected by hand. All values are the mean of at least three measurements on three contiguous cardiac cycles. Cardiac output and fractional shortening were determined 5 min before and at 5 and 10 min after each injection. Heart rate and capillary oxygen levels were determined continuously (Supermon 7210, Kontron Instruments, Elon College, N.C.). The electrocardiographic (ECG) lead 2 was recorded continuously to detect any rhythmic or ischemic changes (Honeywell

VR-12). Fractional shortening was determined according to ACC standards by using the best echocardiographic window, either the left parasternal or the subcostal, using a phased-array system (Ultramark 9 or 7, Advanced Technology Laboratories, Bothell, Wash.) with a 2.25 MHz transducer. The patients were examined in either the supine or lateral decubitus position.

Humoral and Cellular Immunologic Assessments

As stated previously, peripheral blood samples were drawn just before the first Alburnex exposure, and 6.8 ± 1.8 and 31.2 ± 12.2 weeks after the first and the second Alburnex exposures, respectively. Possible effects of Alburnex on the cellular immune system were assessed by lymphocyte transformation test. Peripheral blood lymphocytes were obtained as previously described and cell viability was assessed using the Trypan blue exclusion test.²¹ Thereafter, lymphocytes were divided into three aliquots. The first one was composed of lymphocytes in the presence of Alburnex, at dilutions varying from 1/8 to 1/1024. The two other lymphocytes aliquots were used as controls. Unstimulated lymphocytes allowed verification of the viability of the cells in the medium, and the phytohemagglutinin (PHA)-stimulated lymphocytes, mimicking the *in vivo* activation of T cells by antigen, resulted in blastic transformation as assessed from an increase in ³H-thymidine incorporation. Possible effects of Alburnex on the humoral immune system were examined by assessment of specific IgE and IgG antibodies. Enzyme-linked immunosorbent assays (ELISA) were used to determine whether Alburnex microsphere-specific IgE and IgG antibodies were induced in the patients, each serum sample being tested on microtiter plates coated with washed Alburnex microspheres.

Contrast Agent

Alburnex (Nycomed Imaging AS, Norway; Molecular Biosystems Inc., San Diego, CA) consists of air-filled albumin microspheres suspended in a 5% solution of human albumin; the concentration of sonicated microspheres with a diameter between 4 and 10 microns was 2×10^8 per ml. The product was injected manually at a rate of 1 ml/s in doses of 0.08 and 0.22 ml/kg body weight, followed by a flush with saline for 10 s.

Statistical Analysis

All data are presented as mean $\pm$ SD. The differences between several means were assessed using one-way analysis of variance and, when significant, means were compared two by two using a posteriori Duncan's multiple range test. Changes for one parameter within a group were analyzed by paired *t*-test. Statistical significance was set at $p < 0.05$.

Results

Data concerning the effect of Alburnex on the cellular immune system were pooled for high Alburnex concentrations

(1/8–1/16), corresponding to the expected hepatic concentration of microspheres, and for low concentrations (1/32–1/1024), corresponding to the expected decreasing plasma concentrations (Fig. 1). Compared with the controls with unstimulated lymphocytes, no stimulation of the cellular immune system was observed at any of the Albunex dilutions tested. Conversely, lymphocytes incubated with PHA were stimulated, as inferred from significant increase in ^3H -thymidine incorporation reflecting the blastic transformation.

No effect on the humoral immune system was observed after Albunex injections. Figure 2 demonstrates the lack of specific IgE increase after the first and second exposures to Albunex, as observed with human serum albumin. Similar results were obtained concerning the effect of repeated Albunex on specific IgG. All the values were under the lower limits for positive test for detection of specific antibodies (0.08 for IgE and 0.15 for IgG, paired *t*-test, $p < 0.001$ at all time points).

The hemodynamic characteristics of the patients remained in the normal range. The time course of mean systemic, pulmonary artery, capillary wedge, and right atrial pressures, shown as mean changes from baseline values, are presented in Table I. Eventual changes in cardiac output, fractional shortening, and heart rate are shown in Table II. An increase in mean systemic blood pressure at 10 min after the first injection, a decrease in pulmonary capillary wedge pressure at 3 and 10 min after the first injection and at 10th min after the second injection, and an increase in fractional shortening at 10 min after the second injection were observed. That statistical significance was obtained for changes in these three parameters is not unexpected considering the power of the test used; no change appeared to be of clinical significance.

No significant changes were observed for arterial pH, oxygen and carbon dioxide arterial partial pressures, bicarbonate concentrations, and saturation of hemoglobin by oxygen at any time of measurements.

FIG. 1 Histograms showing the effects of Albunex® on the cellular immune system. Controls (□) were nonstimulated lymphocytes and were compared with lymphocytes growing in the presence of low Albunex dilution (1/32–1/1024, ■), of high Albunex dilution (1/8 to 1/16, ■), and of PHA-stimulated lymphocytes (■). Results are mean + standard deviation. * $p < 0.001$, difference with other group (Duncan's multiple range test).

Discussion

This is the first study reported in instrumented patients, conducted over a period of time long enough to allow the development of an eventual immune reaction, in which the immunologic, clinical, respiratory, and hemodynamic responses to repeated exposure to a clinically useful dose of Albunex have been simultaneously examined.

In humans, as in most animal species, single Albunex exposure is considered to be safe.^{10, 13–16} In animals, nevertheless, repeated Albunex exposure resulted in major adverse effects,^{17, 18} suggesting that repeated injections in humans may be indicated to evaluate the safety of this new agent further. Indeed, although it was likely that these adverse events were the result of sensitization to the human serum albumin fraction, the antigenicity of the sonicated fraction of Albunex was not addressed, leaving the question of an eventual responsibility of the sonication process unanswered.¹⁷

Indeed, Albunex microbubble formation results in the formation of interprotein disulfide bonds.^{11, 12} This chemical cross-linking induces a conformational change in the structural surface of the protein which is commonly accepted as

FIG. 2 Graphs showing the effects of Albunex® on Albunex-specific IgE antibodies in the 10 patients in whom assays were performed before, after first exposure (exp), and after second exposure to Albunex. Serum samples were tested on both microtiter plates coated with human serum albumin (A) or with washed Albunex microspheres (B). The dotted line at 0.08 is the lower limit for positive test for detection of specific IgE antibodies.

TABLE I Hemodynamic effect two successive injections of Alburnex®, performed 4.2 ± 1.5 months after a first Alburnex exposure

	Mean pressures (mmHg)			
	SBP	PAP	PCWP	RAP
Baseline				
–5 min	90.3 ± 16.3	16.5 ± 6.0	11.3 ± 6.3	6.5 ± 4.5
0 min	92.0 ± 17.1	15.6 ± 6.2	10.2 ± 6.3	7.4 ± 4.6
After first injection				
1 min	3.3 ± 5.4	0.1 ± 3.3	–1.5 ± 2.5	–0.5 ± 2.1
3 min	2.8 ± 7.4	–0.6 ± 2.8	–1.7 ± 1.6 ^b	–0.4 ± 1.4
5 min	2.5 ± 4.7	–0.6 ± 2.4	–0.7 ± 2.1	0.4 ± 1.9
10 min	4.3 ± 5.7 ^a	0.5 ± 2.8	–2.0 ± 2.6 ^a	–0.3 ± 1.6
15 min	95.7 ± 25.0	17.8 ± 7.0	10.9 ± 6.3	6.3 ± 4.6
After second injection				
1 min	3.1 ± 6.7	–0.5 ± 2.5	–2.0 ± 3.6	0.5 ± 1.7
3 min	2.4 ± 6.0	–0.8 ± 2.6	–2.0 ± 3.7	0.0 ± 1.2
5 min	1.5 ± 6.4	–0.9 ± 2.8	–1.5 ± 3.7	0.4 ± 1.2
10 min	1.7 ± 4.0	–0.6 ± 1.9	–1.2 ± 1.5 ^a	0.0 ± 1.5

Paired *t*-test: ^a*p* < 0.05, ^b*p* < 0.01.

For baseline and 15 min after injection, the data are mean values ± SD. The other data are expressed as mean changes ± SD (*n* = 12) from baseline, that is, 0 min for first injection, and from 15 min after first injection for the second injection.

Abbreviations: SBP = systemic blood pressure, PAP = pulmonary artery pressure, PCWP = pulmonary capillary wedge pressure, RAP = right atrial pressure, SD = standard deviation.

one of the major determinants of protein antigenicity.¹⁹ Thus, the sonication process may result in the creation of new epitopes in the albumin molecules that results eventually in adverse immunogenic response in recipients.^{22,23} Such stimulation of either the cellular or the humoral immune system may lead to dramatic adverse effects.^{24–26}

Our data showed that while the viability and reactivity of the patients' lymphocytes were normal as inferred from the PHA-stimulated blastic transformation, no Alburnex-induced blastic transformation of patients' lymphocytes was observed in the low-concentration group. This concentration was likely to mimic that observed in blood, where the Alburnex concentration decreases steadily through dilution in the blood volume. Nevertheless, Alburnex microspheres are taken up by the

liver where "shells" are concentrated.²⁷ Thus, one can postulate that only very high concentrations of Alburnex might induce blastic transformation of patients' lymphocytes. To mimic the high Alburnex concentration likely to be observed in the human liver, where Kupfer cells are numerous, we performed transformation assays using 1/8 to 1/16 in vitro Alburnex dilutions. Again, our data revealed no blastic transformation. Thus, no adverse effect on the cellular immune system was observed after repeated intravenous Alburnex exposure, regardless of the Alburnex dilution tested.

Similarly, we observed no formation of microsphere-specific IgE and IgG antibodies after either the first or the second exposure to Alburnex in our patients. Our results suggest an absence of adverse effects of such a transpulmonary echocontrast agent on the cellular and humoral immune system in patients. These data are in agreement with those from a previous study in normal humans showing the lack of skin reactions and IgE secretion in response to repeated exposure to Alburnex.²⁸ Indeed, no specific IgE or IgG secretion was observed in 17 normal volunteers after four intravenous injections, at 4-week intervals, of low doses of Alburnex.²⁹ Consequently, the antigenicity of the sonicated fraction of Alburnex is probably low. However, skin testing has been reported to be negative in the case of a documented anaphylactoid reaction to intravenous administration of 99m-Tc-labeled human albumin microspheres.³⁰ Moreover, it is widely accepted that although useful, no prognostic test is available to identify with certainty patients at high risk of reaction.²⁰

These limitations mean that caution must be applied when interpreting immunologic results alone and suggest that invasive hemodynamic monitoring may be useful, particularly since mild clinical reactions might otherwise have been missed. The invasive monitoring performed in our patients showed no significant alteration in right and left heart hemodynamics. Indeed, although statistically significant, the variations observed in some hemodynamic values were slight and probably not of clinical importance, particularly with respect to indicating an anaphylactoid type reaction. Furthermore, no deleterious effect was found on respiratory function, and arterial blood gases were not significantly modified throughout the study. Although the H₁ receptors antagonist, given to nine of our patients for its anxiolytic properties, may eventually have diminished clinical signs, our data suggest a lack of ana-

TABLE II Effect of repeated exposure to Alburnex®: time course of possible changes in cardiac output (CO), fractional shortening (FS), and heart rate (HR) after two successive injections of Alburnex, performed 4.2 ± 1.5 months after a first Alburnex exposure

	Baseline	After first injection		After second injection	
	–5 min	5 min	10 min	5 min	10 min
CO (l/min)	5.1 ± 1.1	0.0 ± 0.6	–0.1 ± 0.5	–0.1 ± 0.6	–0.1 ± 0.6
FS (%)	29.5 ± 7.0	1.6 ± 2.7	0.3 ± 3.6	1.2 ± 3.1	2.4 ± 2.5 ^a
HR (beats/min)	63.1 ± 15.4	1.3 ± 5.4	2.8 ± 8.4	0.8 ± 4.3	0.2 ± 3.4

^aPaired *t*-test, *p* < 0.02.

Data are expressed as mean changes from baseline ± standard deviation (*n* = 12). Each parameter was determined 5 min before and 5 and 10 min after each injection.

phylactoid response to repeated Alburnex in our patients. Indeed, anaphylactoid reactions may occur without any plasma histamine elevation. Furthermore, even if such treatment may blunt an anaphylactoid type reaction, histamine-induced adverse effect would not have been missed by continuous invasive right and left heart monitoring.²⁰

Conclusion

These results show that repeated exposure to a clinically useful dosage of Alburnex, in a group of patients who will presumably benefit the most from multiple contrast echocardiographic studies through myocardial perfusion assessment and contrast-added stress echocardiographic examinations, did not induce adverse effects in either the cellular or humoral immune system. Moreover, no deleterious effects were observed on clinical status, left and right heart hemodynamics, or respiratory function of the patients. They suggest that although anaphylactoid type reactions against aggregates of albumin have been previously reported,³⁰ the risk of repeated exposure to Alburnex in humans may be minimal as inferred from previous experience with human albumin-containing products.³¹ Further studies on larger population samples may therefore need to be performed to confirm that such risk may not be higher than for repeated injections of human albumin solution, allowing a broader use of such a transpulmonary echocontrast agent.

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