Effect of pulse polarity and energy on ultrasound-induced lung hemorrhage in adult rats

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The objective of this study was to further assess the role of inertial cavitation in ultrasound-induced lung hemorrhage by examining the effect of pulse polarity at a common *in situ* (at the lung surface) peak rarefactional pressure [$p_{r(in\ situ)}$] and at a common *in situ* pulse intensity integral ($\mathrm{PII}_{in\ situ}$). A total of 60 rats was divided into three experimental groups of 20 animals per group and randomly exposed to pulsed ultrasound. The groups were exposed as follows: Group 1 to 0° polarity pulses (compression followed by rarefraction) at a $p_{r(in\ situ)}$ of 3.48 MPa and a $\mathrm{PII}_{in\ situ}$ of 4.78 Ws/m^2, group 2 to 180° polarity pulses (rarefraction followed by compression) at a $p_{r(in\ situ)}$ of 3.72 MPa and a $\mathrm{PII}_{in\ situ}$ of 2.55 Ws/m^2, and group 3 to 180° polarity pulses at a $p_{r(in\ situ)}$ of 4.97 MPa and a $\mathrm{PII}_{in\ situ}$ of 4.79 Ws/m^2. For all experimental groups, the frequency was 2.46 MHz, the exposure duration was 240 s, the pulse repetition frequency was 2.5 kHz, and the pulse duration was 0.42 μs. Six sham animals were also randomly distributed among the experimental animals. The lesion surface area and depth were determined for each rat as well as lesion occurrence (percentage of rats with lesions) per group. It was found that lesion occurrence and size correlated better with $\mathrm{PII}_{in\ situ}$ than with $p_{r(in\ situ)}$, suggesting that a mechanism other than inertial cavitation was responsible for the damage. © *2003 Acoustical Society of America.* [DOI: 10.1121/1.1559176]

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I. INTRODUCTION

Several research groups have observed ultrasonically produced hemorrhage in the lung. These groups have also been interested in the mechanism of damage.[1–20] Several studies have indicated that heating is not responsible for ultrasound-induced lung hemorrhage,[1,21] and there is agreement that gas in the lung plays a role in ultrasound-induced damage.[2,16] Even though the aerated lung requirement suggests cavitation as the mechanism responsible for lung damage, a distinction must be made between mechanisms involving large gas bodies, such as gas in the alveoli of the lung (38–49 μm[22–24]), and classical inertial cavitation that involves small microbubbles as nuclei (radii on the order of 1 μm or less[25]). Evidence has been slowly accumulating that suggests that the mechanism of damage in the lung may not be inertial cavitation.[17,26,27] There seems to be no dependence on whether the negative or positive pressure components of the ultrasonic pulse cause lithotripter-induced lung damage; however, inertial cavitation is associated with the negative pressure.[28] The frequency dependence may not be the same as that associated with effects due to the presence of contrast agents that quite clearly nucleate inertial cavitation.[29] The hydrostatic pressure dependence of ultrasound-induced lung hemorrhage in mice is not the same as that associated with effects due to inertial cavitation.[17] Albunex does not seem to increase the sensitivity of the lung to pulsed ultrasound.[30] However, there is evidence that suggests the mechanism of damage in rat lung may be inertial cavitation,[10,31] and at least one investigator argues that previously reported studies using overpressure[17] may not conclusively demonstrate that inertial cavitation is not responsible.[32–35]

This study has been designed to further investigate the possible role of inertial cavitation in lung hemorrhage. The peak negative pressure is known to be associated with the onset of inertial cavitation. This fact was the basis for including $p_{r.3}$, the water-based value of the peak rarefactional pressure derated by 0.3 dB/cm-MHz, in the definition of the mechanical index (MI). The MI is the output display quantity intended to indicate the relative likelihood of mechanically induced biological effects associated with an ultrasound examination. Further, it has been shown theoretically that the threshold for inertial cavitation is lower when the rarefaction portion of a pulse precedes the compression portion, rather than when the sequence is reversed.[36–38]

Flynn[36] showed theoretically that there were differences in the temporal response of the bubble radius and the quan-

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TABLE I. Exposimetry quantities. The pulse duration, ultrasonic frequency, and exposure duration for all exposures were 0.42 μs, 2.46 MHz, and 240 s, respectively. For the shams, the pulse repetition frequency was 10 Hz, and for the three experimental groups, the pulse repetition frequency was 2.5 kHz.

Group	Polarity (degrees)	$p_{r(in\ situ)}$ (MPa)	$p_{c(in\ situ)}$ (MPa)	$\mathrm{PII}_{in\ situ}$ (Ws/m^2)	$I_{\mathrm{TA}(in\ situ)}$ (W/cm^2)	MI
Shams	0	0.15	0.18	0.0044	4.4×10^{-6}	0.070
1	0	3.48	9.05	4.78	1.20	1.60
2	180	3.72	4.23	2.55	0.64	1.75
3	180	4.97	6.80	4.79	1.20	2.31

tities associated with bubble collapse depending upon polarity of the pulse. He showed that for the 180° polarity pulse (rarefaction followed by compression) the maximum values for the collapse pressure, $P_{\max}$, and temperature, $T_{\max}$, within the bubble were greater than for the 0° polarity pulse (compression followed by rarefaction), i.e., $P_{\max}=9$ kbar [900 MPa] versus 4.2 kbar [420 MPa], and $T_{\max}=4775$ K versus 3105 K for an acoustic pulse of amplitude 3 bar [0.3 MPa] incident on a 1-μm-radius bubble in water. However, the maximum bubble radius achieved was slightly greater for the 0° polarity pulse. Flynn[36] attributed the lower values of $P_{\max}$ and $T_{\max}$ associated with a 0°-polarity acoustic pulse that gave a larger maximum bubble radius to the ''absence of a positive pressure peak that can do appreciable work on the expanded cavity.''

Consistent with this hypothesis is the work of Apfel and Holland,[39,40] who explored theoretically the threshold for inertial cavitation associated with a single-cycle acoustic pulse. They considered only a pulse that had a rarefaction preceding a compression (180° polarity pulse), clearly implying that they expected this sequence would yield the lowest threshold for inertial cavitation.

Morgan *et al.*[37,38] showed theoretically and experimentally that the acoustic echo amplitude from encapsulated gas contrast agents was greater from incident 180° polarity pulses than from incident 0° polarity pulses. Also, the initiation of the echo pulse coincided with the negative portion of the incident pulse in both cases. Thus, the echo pulse associated with the 0°-polarity pulse did not begin with the initial compression portion of the incident pulse but was delayed until occurrence of the rarefaction portion of the incident pulse. For the 180°-polarity pulse the rarefactional portion of the incident waveform is followed by a compression, which results in a higher bubble wall velocity during collapse and a higher center frequency for the echo. Thus, experimentally and theoretically the 180°-polarity pulse gave a greater response.

The first hypothesis tested for the study reported herein was that inertial cavitation is not responsible for ultrasound-induced lung hemorrhage. To test this hypothesis two different temporal pulse waveforms with the same value of $p_{r(in\ situ)}$ were used, and will be referred to as the 180° (rarefaction followed by compression) and 0° (compression followed by rarefaction) polarity pulses. If this hypothesis were supported, then the 180° polarity pulse would not produce a greater effect on lung than the 0°-polarity pulse. This represents the first test of the effects of pulse polarity on lung hemorrhage with diagnostic-like ultrasound pulses.

Another aspect of this study was to examine the role of the energy associated with the ultrasound exposure. In previous studies[41,27] the energy incident on the lung had been shown to relate to lesion occurrence and lesion size under superthreshold conditions. Thus, we designed this study so that we could also test a second hypothesis, which was that superthreshold lung hemorrhage would correlate with the value of the $\mathrm{PII}_{in\ situ}$ or energy associated with the exposure. To test this hypothesis both the 180°- and 0°-polarity waveforms were used, except that the value of $\mathrm{PII}_{in\ situ}$ was held constant. If this hypothesis were supported, then the effect on the lung would be similar for the two pulses.

II. MATERIALS AND METHODS

A. Exposimetry

Ultrasonic exposures were conducted using a focused, 2.54-mm-diameter, PZT ultrasonic transducer (Valpey Fisher, Hopkinton, MA). Water-based (degassed water, 22 °C) pulse-echo ultrasonic field distribution measurements were performed according to established procedures,[42,19] and yielded a center frequency of 2.46 MHz, a fractional bandwidth of 38%, a −6-dB beamwidth of 1.54 mm, a −6-dB depth of focus of 14.4 mm, and a focal length of 39 mm. An automated procedure was used to routinely calibrate the ultrasound fields[43,44,19] (Table I) that was based on established standards.[45,46] Briefly, the source transducer's drive voltage was supplied by a RAM5000 (Ritec, Inc., Warwick, RI). A calibrated PVDF membrane hydrophone (Marconi model Y-34-6543, Chelmsford, UK) was mounted to the computer-controlled micropositioning system (Daedal, Inc., Harrisburg, PA). The hydrophone's signal was digitized with an oscilloscope (500 Ms/s, LeCroy model 9354TM, Chestnut Ridge, NY), the output of which was fed to the same computer (Dell Pentium II, Dell Corporation, Round Rock, TX) that controlled the positioning system. Off-line processing (MATLAB, The Mathworks, Natick, MA) yielded the peak water-based rarefactional pressure $p_{r(in\ vitro)}$, the peak water-based compressional pressure $p_{c(in\ vitro)}$, and the water-based pulse intensity integral ($\mathrm{PII}_{in\ vitro}$). The temporal-average intensity (I_{TA}) at the focus was calculated. The mechanical index (MI) was also determined.[45] The MI is reported because it is a regulated quantity[47,48] of diagnostic ultrasound systems, and its value is available to system operators. Thus, there is value to provide the MI for each of our exposure settings in order to give general guidance to manu-

facturers and operators as to the levels we are using in this study. Further, it is a quantity that cannot be determined directly from $p_{r(in\ situ)}$.

The two different polarity pulses were obtained by changing the polarity of the applied electrical signal; examples of the recorded acoustic waveforms measured in water are shown in Fig. 1. It is readily seen that these waveforms contain more than one cycle. As expected for experimental sources the pulse increases in amplitude to a maximum and then decreases, in this case including on the order of three complete cycles. This necessitates a very clear definition of the 0°- and 180°-polarity waveforms used in this study. The 0°-polarity waveform is defined as one having the first of the two largest half-cycles as a compression, while the 180°-polarity waveform has the first of the two largest half-cycles as a rarefaction. This is entirely consistent with the definition of 0°- and 180°-polarity pulses as used by Morgan *et al.*[38] for their pulses that consisted of approximately two complete cycles.

Fourteen independent calibrations were performed weekly during the time period of the experiment. One set of calibrations was performed before exposures were initiated each week and one set of calibrations was performed after exposures were concluded for each week. Relative standard deviations (standard deviation/mean) of $p_{r(in\ vitro)}$ and $p_{c(in\ vitro)}$ were 6% and 9%, respectively. The relative standard deviation of $\mathrm{PII}_{in\ vitro}$ was 11%.

The *in situ* (at the pleural surface) peak rarefactional pressures, peak compressional pressures, and pulse intensity integrals were estimated from their respective *in vitro* values, the mean attenuation coefficient of the chest wall's intercostal tissue (2.5 dB/cm at 2.46 MHz),[49] and the mean chest-wall thickness (66 rats: 4.76 ± 0.11 mm). The experimental findings were analyzed and reported in terms of the *in situ* peak rarefractional pressure $p_{r(in\ situ)}$, *in situ* peak compressional pressure $p_{c(in\ situ)}$, *in situ* pulse intensity integral $\mathrm{PII}_{in\ situ}$, the temporal-average intensity $I_{\mathrm{TA}(in\ situ)}$, and mechanical index MI (Table I).

B. Animals

The experimental protocol was approved by the campus' Laboratory Animal Care Advisory Committee and satisfied all campus and National Institutes of Health rules for the humane use of laboratory animals. Animals were housed in an AAALAC-approved animal facility, placed in groups of three or four in polycarbonate cages with beta-chip bedding and wire bar lids, and provided food and water *ad libitum*. The AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care, Rockville, MD) is a private nonprofit organization that promotes the humane treatment of animals in science through a voluntary accreditation program.

A total of 60 10- to-11-week-old 327 ± 22-gram female Sprague-Dawley rats (Harlan, Indianapolis, IN) were assigned to one of three experimental groups at random (Table I). An additional six rats were assigned as shams and incorporated into the randomized design. All experimental animals were exposed to 2.46-MHz ultrasound with a 2.5-kHz pulse repetition frequency and a 240-s exposure duration.

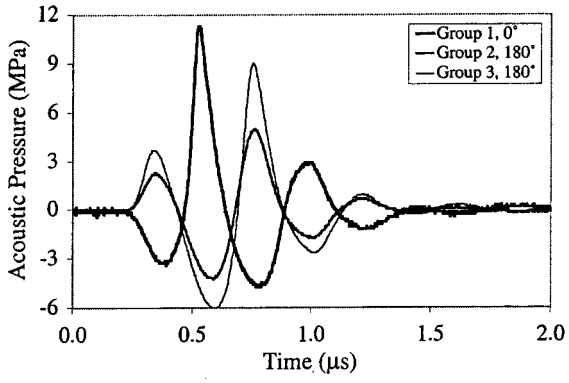

FIG. 1. Measured ultrasonic pressure waveforms in water for the three experimental groups: Group 1, 0° polarity, $p_{r(in\ situ)} = 3.48$ MPa, $\mathrm{PII}_{in\ situ} = 4.78$ Ws/m^2; group 2, 180° polarity, $p_{r(in\ situ)} = 3.72$ MPa, $\mathrm{PII}_{in\ situ} = 2.55$ Ws/m^2; and group 3, 180° polarity, $p_{r(in\ situ)} = 4.97$ MPa, $\mathrm{PII}_{in\ situ} = 4.79$ Ws/m^2.

Experimental group 1 consisted of animals exposed with 0°-polarity pulses and served as the baseline comparison group [$p_{r(in\ situ)} = 3.48$ MPa and $\mathrm{PII}_{in\ situ} = 4.78$ Ws/m^2]. The other two groups were both exposed with pulses of polarity 180°. Group 2 was designed to have the same peak rarefactional pressure [$p_{r(in\ situ)} = 3.72$ MPa and $\mathrm{PII}_{in\ situ} = 2.55$ Ws/m^2] as group 1, and group 3 was designed to have the same pulse intensity integral [$p_{r(in\ situ)} = 4.97$ MPa and $\mathrm{PII}_{in\ situ} = 4.79$ Ws/m^2] as group 1. The actual values for quantities designed to be the same vary slightly because of variation in the mean chest-wall thickness among the groups. Sample waveforms measured in water are shown in Fig. 1 for the three experimental groups. The baseline exposure for group 1 was chosen to be at a superthreshold level corresponding to approximately 50% lesion occurrence based on previous results,[18,19,49] so that changes in lesion occurrence and size could be easily compared among the groups.

Rats were weighed and then anesthetized with ketamine hydrochloride (87.0 mg/kg) and xylazine (13.0 mg/kg) administered intraperitoneally. The skin of the left thorax was exposed by removing the hair with an electric clipper, followed by a depilatory agent (Nair® Carter-Wallace, Inc., New York, NY) to maximize sound transmission. A black dot was placed on the skin over the ribs at approximately the sixth to ninth rib to guide the positioning of the ultrasonic beam. Anesthetized animals were placed in a specially designed holder to which the ultrasonic transducer was attached. A removable pointer, attached to the transducer, was used to position the ultrasonic beam perpendicular to the skin at the position of the black dot with the beam's focal region approximately at the lung surface.[19] The ultrasonic beam was incident on the lateral surface of the lung.

The holder with the animal and mounted transducer was placed in highly degassed, temperature-controlled (30 °C) water. The low-power pulse-echo capability of the RAM5000 exposure system displayed on an oscilloscope was used to adjust the axial center of the focal region to within 1 mm of the lung surface. It was during this part of the experimental procedure that the 13-ohm in-line attenuator was placed between the RAM5000 and transducer to obtain very low exposure values (see the row ''shams'' in

TABLE II. Percentage of animals with lesions and means (±s.d.) for lesion depth, surface area, and volume for shams and experimental groups.

Group	Number of animals	Lesion occurrence (percent)	Lesion depth (mm)	Lesion surface area (mm^2)	Lesion volume (mm^3)
Shams	6	17	0.18 (±0.43)	0.62 (±1.5)	0.22 (±0.54)
1	20	50	0.49 (±0.53)	1.58 (±2.4)	0.57 (±0.99)
2	20	10	0.08 (±0.24)	0.14 (±0.49)	0.04 (±0.13)
3	20	60	0.64 (±0.85)	2.29 (±3.0)	1.23 (±0.99)

Table I for these low-level ultrasonic pressure levels). Also, the pulse repetition frequency was reduced to 10 Hz during this alignment procedure. The ultrasound propagation medium between the transducer and the animal's skin surface was highly degassed water, as was used for transducer calibrations. Animals were exposed to pulsed ultrasound with a pulse repetition frequency of 2.5 kHz and an exposure duration of 240 s. Following exposure, rats were removed from the water and holder, and euthanized under anesthesia by cervical dislocation.

The left thoracic wall was opened and the thickness of the intercostal tissue (skin, fat, fascia, muscle, and parietal pleura) between the ribs was measured with a digital micrometer (accuracy: 10 μm) at the black dot used for transducer alignment. These chest-wall measurements were used for later calculation of the *in situ* ultrasonic pressures at the visceral pleural surface. The lungs were removed from each animal and the left lung was scored for the presence or absence of hemorrhage. As previously reported,[17,19] and also observed in this study, lung hemorrhage formed along the pathway of the ultrasound beam and the lesion assumed a conical shape. The base of the lesion originated at the visceral pleural surface and was elliptical in shape. The lesion extended into lung parenchyma to form its apex at varied depths within the lung. The left lung was fixed by immersion in 10% neutral-buffered formalin for a minimum of 24 h. After fixation, the elliptical dimensions of each lung lesion at the visceral pleural surface were measured using a digital micrometer where ''a'' is the semimajor axis and ''b'' is the semiminor axis. The lesion was then bisected and the depth ''d'' of the lesion within the pulmonary parenchyma was also measured. The surface area (πab) and volume (πabd/3) of the lesion were calculated for each animal. Each half of the bisected lesion was embedded in paraffin, sectioned at 5 μm, stained with hematoxylin and eosin, and evaluated microscopically.

C. Statistics

The lesions' depth, surface area, and volume were compared among the groups to determine if differences were significant. Groups were determined to be significantly different at the 0.05 level if the ranges defined by their (mean) ± 1.96× (standard error of the mean) did not overlap. Otherwise the differences were not significant.

III. RESULTS

The fraction of animals with lesions was determined for the shams and each of the three experimental groups (Table II and Figs. 2 and 3). The fraction of animals with hemorrhage in the shams was 17% (one out of six rats) and for groups 1, 2, and 3 was 50%, 10%, and 60% (each out of 20 rats), respectively.

The means and standard deviations for depth, surface area, and volume of the lesions for the shams and groups 1, 2, and 3 are shown in Table II and Fig. 3. Although the lesion volume was not measured directly, it was calculated from the surface area and the depth of the lesion and is provided here for completeness. For all three lesion size quantities, there was a statistically significant difference between groups 1 and 2, but no significant difference between groups 1 and 3.

IV. DISCUSSION

Two hypotheses related to the determination of the mechanism for lung hemorrhage were tested in this study. The first hypothesis was that ultrasound-induced lung hemorrhage is not caused by inertial cavitation. The threshold for inertial cavitation depends upon peak rarefactional pressure, and both theoretical and experimental results predict that quantities associated with bubble collapse (maximum collapse pressure, maximum collapse temperature, and wall speed during collapse) are greater when the rarefactional portion of a pulse precedes the compressional portion of the pulse. These theoretical and experimental observations were

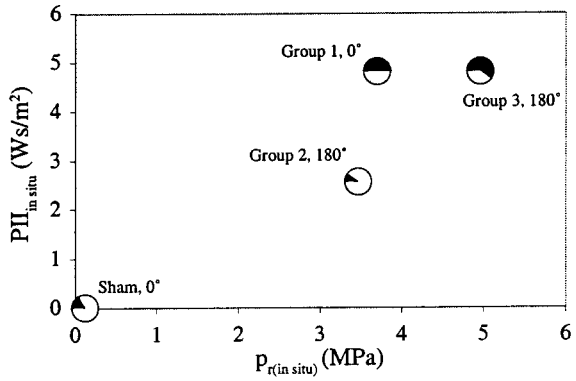

FIG. 2. Fraction of animals with lesions versus $p_{r(in\ situ)}$ and $PII_{in\ situ}$ for sham and experimental groups. The fraction of the circles colored black represents the fraction of animals with lesions.

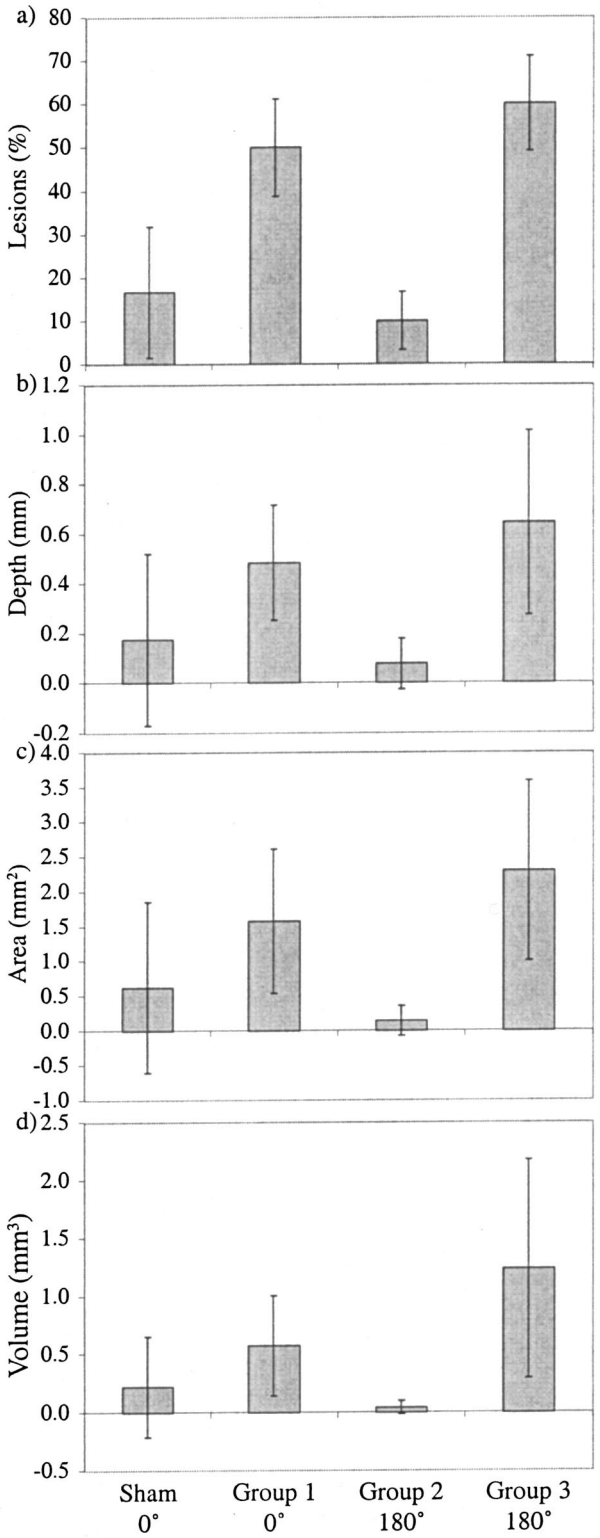

FIG. 3. Lesion (a) occurrence; (b) depth; (c) surface area; and (d) volume for sham and experimental groups.

the basis for the design of two of the experimental groups in this study. Experimental groups 1 and 2 were designed to have the same peak rarefactional pressure; one group had the compressional portion first (group 1, 0°) and the other had the rarefactional portion first (group 2, 180°). The actual peak rarefactional pressures were slightly different for the two groups because the mean chest-wall thicknesses differed. Note that the largest two pulses in the waveform were used to determine whether the rarefactional peak was second or first and that this was consistent with the definition of 0° and 180° pulses, respectively, as used by Morgan *et al.*[38] If inertial cavitation was the primary mechanism responsible for lesion production, then the lesion percentage for group 2 should have been greater than that for group 1. Instead, exactly the opposite occurred (Table II and Figs. 2 and 3), which supports the hypothesis by providing direct evidence that inertial cavitation was not the primary mechanism responsible for the lung damage.

The second hypothesis tested was that the lung hemorrhage would correlate with the value of the $PII_{in\ situ}$ or energy associated with the exposure. In previous studies,[41,27] the energy incident on the lung had been shown to relate to lesion occurrence and lesion size under superthreshold conditions. Thus, another experimental group (group 3, $PII_{in\ situ}=4.79\ Ws/m^2$) was established to have the same pulse intensity integral as group 1 ($PII_{in\ situ}=4.78\ Ws/m^2$). Again, there was a very small difference in the value of the pulse intensity integral between the two groups due to chest-wall thickness differences. Because the transducer, frequency, pulse repetition frequency, and exposure duration were the same for all three groups, the power and energy incident on the lung surface for these two groups were proportional to $PII_{in\ situ}$. The results show that lesion occurrence was nearly the same for these two groups (group 1 = 50% and group 3 = 60%). This result supported the second hypothesis and lends even further experimental evidence that inertial cavitation was not involved because lesion occurrence at these superthreshold conditions correlated better with incident energy than with peak rarefactional pressure.

Similar results were observed when lesion depth, surface area, and volume were examined. For all three of these lesion size quantities, there were statistically significant differences between groups 1 and 2, but no significant differences between groups 1 and 3 (Table II and Fig. 3).

The pulses used in this study consisted not of a simple single cycle or half-cycle but of multiple cycles that were much more representative of diagnostic pulses. In this respect, they were similar to pulses used by Morgan *et al.*[37,38] and had the advantage of mimicking real-world ultrasound exposures. However, they had the disadvantage that as a result of transducer response and nonlinear propagation, the pulses for the three groups differed in ways other than simply the pulse polarity and amplitude adjustment for common peak rarefactional pressure or common pulse intensity integral. For example, the peak compressional pressure was greater for groups 1 and 3 than for group 2. However, this difference also supported the conclusion that inertial cavitation was not responsible for the lung hemorrhage because inertial cavitation is not associated with the peak compressional pressure.

This analysis is of course not complete without considering the sham exposed animals. One of the six sham animals used in this study had a lesion. In the last several years, we have conducted experiments involving exposure of the lung in approximately 1500 rats. Each of these experiments involved shams and we have used approximately 150 rats as

shams. Prior to this study in no case has there ever been any lesions found in the shams. That previous experience and the fact that the lesion in the one sham animal reported herein was quite large leads us to believe that this lesion is an outlier. Even though we are convinced that is the case, the results for this animal had to be included with our analysis. The sham exposed animals exhibited a lesion occurrence and lesion dimensions that were greater than in group 2, but smaller than groups 1 and 3 (Table II and Figs. 2 and 3). Statistical comparisons between any of the experimental groups and the shams have limited meaning because only one sham animal had a lesion, leading to a very large standard error of the mean and no real statistical significance between the shams and any of the three experimental groups. This in no way lessens the statistical comparisons among the experimental groups that were presented above.

Finally, the MI was developed to provide an estimate of the potential for mechanically induced bioeffects,[45] and is given by

$$\mathrm{MI}=\frac{p_{r.3}}{\sqrt{f}}, \tag{1}$$

where $p_{r.3}$ is the water-based peak rarefactional pressure derated by 0.3 dB/cm-MHz at the location where the derated pulse intensity integral $\mathrm{PII}_{.3}$ is a maximum and f is the ultrasonic frequency in megahertz. The MI measurement procedure[45] was that used to determine the MI reported herein (Table I). The frequency dependence built into the MI was developed specifically to reflect the best idea as to the dependence of the likelihood for inertial cavitation on frequency under specified conditions.[16,40,45] The results of this study provide further evidence that the MI is not a good predictor of lung hemorrhage.

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[1] S. Z. Child, C. L. Hartman, L. A. Schery, and E. L. Carstensen, "Lung damage from exposure to pulsed ultrasound," Ultrasound Med. Biol. **16**, 817–825 (1990).
[2] C. Hartman, S. Z. Child, R. Mayer, E. Schenk, and E. L. Carstensen, "Lung damage from exposure to the fields of an electrohydraulic lithotripter," Ultrasound Med. Biol. **16**, 675–679 (1990).
[3] D. P. Penney, E. A. Schenk, K. Maltby, C. Hartman-Raeman, S. Z. Child, and E. L. Carstensen, "Morphologic effects of pulsed ultrasound in the lung," Ultrasound Med. Biol. **19**, 127–135 (1993).
[4] C. H. Raeman, S. Z. Child, and E. L. Carstensen, "Timing of exposures in ultrasonic hemorrhage of murine lung," Ultrasound Med. Biol. **19**, 507–517 (1993).
[5] L. A. Frizzell, E. Chen, and C. Lee, "Effects of pulsed ultrasound on the mouse neonate: Hind limb paralysis and lung hemorrhage," Ultrasound Med. Biol. **20**, 53–63 (1994).
[6] C. K. Holland, K. Sandstrom, X. Zheng, J. Rodriguey, and R. A. Roy, "The acoustic field of a pulsed Doppler diagnostic ultrasound system near a pressure-release surface," J. Acoust. Soc. Am. **95**, 2855(A) (1994).
[7] A. F. Tarantal and D. R. Canfield, "Ultrasound-induced lung hemorrhage in the monkey," Ultrasound Med. Biol. **20**, 65–72 (1994).
[8] J. F. Zachary and W. D. O'Brien, Jr., "Lung lesions induced by continuous- and pulsed-wave (diagnostic) ultrasound in mice, rabbits, and pigs," Vet. Pathol. **32**, 43–54 (1995).
[9] R. Baggs, D. P. Penney, C. Cox, S. Z. Child, C. H. Raeman, D. Dalecki, and E. L. Carstensen, "Thresholds for ultrasonically induced lung hemorrhage in neonatal swine," Ultrasound Med. Biol. **22**, 119–128 (1996).
[10] C. K. Holland, C. X. Deng, R. E. Apfel, J. L. Alderman, L. A. Ferandez, and K. J. W. Taylor, "Direct evidence of cavitation *in vivo* from diagnostic ultrasound," Ultrasound Med. Biol. **22**, 917–925 (1996).
[11] C. H. Raeman, S. Z. Child, D. Dalecki, C. Cox, and E. L. Carstensen, "Exposure-time dependence of the threshold for ultrasonically induced murine lung hemorrhage," Ultrasound Med. Biol. **22**, 139–141 (1996).
[12] D. Dalecki, S. Z. Child, C. H. Raeman, C. Cox, D. P. Penny, and E. L. Carstensen, "Age dependence of ultrasonically induced lung hemorrhage in mice," Ultrasound Med. Biol. **23**, 767–776 (1997).
[13] D. Dalecki, S. Z. Child, C. H. Raeman, C. Cox, and E. L. Carstensen, "Ultrasonically induced lung hemorrhage in young swine," Ultrasound Med. Biol. **23**, 777–781 (1997).
[14] W. D. O'Brien, Jr. and J. F. Zachary, "Lung damage assessment from exposure to pulsed-wave ultrasound in the rabbit, mouse, and pig," IEEE Trans. Ultrason. Ferroelectr. Freq. Control **44**, 473–485 (1997).
[15] WFUMB Symposium on Safety of Ultrasound in Medicine: Issues and Recommendations Regarding Non-Thermal Mechanisms for Biological Effects of Ultrasound [Ultrasound Med. Biol. **24**, Supplement 1, S1–S55 (1998)].
[16] *Mechanical Bioeffects from Diagnostic Ultrasound: AIUM Consensus Statements* (American Institute of Ultrasound in Medicine, Laurel, MD, 1998). Also, J. Ultrasound Med. **19**, 67–168 (2000).
[17] W. D. O'Brien, Jr., L. A. Frizzell, R. M. Weigel, and J. F. Zachary, "Ultrasound-induced lung hemorrhage is not caused by inertial cavitation," J. Acoust. Soc. Am. **108**, 1290–1297 (2000).
[18] W. D. O'Brien, Jr., L. A. Frizzell, D. J. Schaeffer, and J. F. Zachary, "Superthreshold behavior of ultrasound induced lung hemorrhage in adult mice and rats: Role of pulse repetition frequency and pulse duration," Ultrasound Med. Biol. **27**, 267–277 (2001).
[19] J. F. Zachary, J. M. Sempsrott, L. A. Frizzell, D. G. Simpson, and W. D. O'Brien, Jr., "Superthreshold behavior and threshold estimation of ultrasound-induced lung hemorrhage in adult mice and rats," IEEE Trans. Ultrason. Ferroelectr. Freq. Control **48**, 581–592 (2001).
[20] J. F. Zachary, L. A. Frizzell, K. S. Norrell, J. P. Blue, Jr., R. J. Miller, and W. D. O'Brien, Jr., "Temporal and spatial evaluation of lesion reparative responses following superthreshold exposure of rat lung to pulsed ultrasound," Ultrasound Med. Biol. **27**, 829–839 (2001).
[21] C. L. Hartman, S. Z. Child, D. P. Penney, and E. L. Carstensen, "Ultrasonic heating of lung tissue," J. Acoust. Soc. Am. **91**, 513–516 (1992).
[22] M. L. Crosfill and J. G. Widdicombe, "Physical characteristics of the chest and lungs and the work of breathing in different mammalian species," J. Physiol. (London) **158**, 1–14 (1961).
[23] S. M. Tenney and J. E. Remmers, "Comparative quantitative morphology of the mammalian lung: Diffusing area," Nature (London) **197**, 54–56 (1963).
[24] E. R. Weibel, "Dimensions of the tracheobronchial tree and alveoli," in *Biological Handbooks: Respiration and Circulation*, edited by P. L. Altman and D. S. Dittmer (Federation of American Societies for Experimental Biology, Bethesda, MD), Chap. 51.
[25] H. G. Flynn and C. C. Church, "Transient pulsations of small gas bubbles in water," J. Acoust. Soc. Am. **84**, 985–998 (1988).
[26] E. L. Carstensen, S. Gracewski, and D. Dalecki, "The search for cavitation *in vivo*," Ultrasound Med. Biol. **26**, 1377–1385 (2000).
[27] W. D. O'Brien, Jr., J. M. Kramer, T. G. Waldrop, L. A. Frizzell, and J. F. Zachary, "Ultrasound-induced lung hemorrhage: Role of acoustic boundary conditions at the pleural surface," J. Acoust. Soc. Am. **111**, 1102–1109 (2002).
[28] M. R. Bailey, D. Dalecki, S. Z. Child, C. H. Raeman, D. P. Penney, D. T. Blackstock, and E. L. Carstensen, "Bioeffects of positive and negative acoustic pressures *in vivo*," J. Acoust. Soc. Am. **100**, 3941–3946 (1996).
[29] E. L. Carstensen, D. Dalecki, S. M. Gracewski, and T. Christopher, "Nonlinear propagation and the output indices," J. Ultrasound Med. **18**, 69–80 (1999).
[30] C. H. Raeman, D. Dalecki, S. Z. Child, R. S. Meltzer, and E. L. Carstensen, "Albunex does not increase the sensitivity of the lung to pulsed ultrasound," Echocardiography **14**, 553–557 (1997).
[31] C. K. Holland, R. A. Roy, R. W. Biddinger, C. J. Disimile, and C. Cawood, "Cavitation mediated rat lung bioeffects from diagnostic ultrasound," J. Acoust. Soc. Am. **109**, 2433(A) (2001).
[32] R. E. Apfel, Comment on "Ultrasound-induced lung hemorrhage is not caused by inertial cavitation," J. Acoust. Soc. Am. **110**, 1737 (2001).

[33] R. E. Apfel, ''Reply to Frizzell *et al.*'s comment to our comment,'' J. Acoust. Soc. Am. **110**, 1740 (2001).
[34] L. A. Frizzell, J. M. Kramer, J. F. Zachary, and W. D. O'Brien, Jr., Response to ''Comment on 'Ultrasound-induced lung hemorrhage is not caused by inertial cavitation,'' J. Acoust. Soc. Am. **110**, 1738–1739 (2001).
[35] L. A. Frizzell, J. M. Kramer, J. F. Zachary, and W. D. O'Brien, Jr., ''Comment on Apfel's second comment,'' J. Acoust. Soc. Am. **110**, 1742 (2001).
[36] H. G. Flynn, ''Generation of transient cavities in liquids by microsecond pulses of ultrasound,'' J. Acoust. Soc. Am. **72**, 1926–1932 (1982).
[37] K. Morgan, M. Averkiou, and K. Ferrara, ''The effect of the phase of transmission on contrast agent echoes,'' IEEE Trans. Ultrason. Ferroelectr. Freq. Control **45**, 872–875 (1998).
[38] K. E. Morgan, J. S. Allen, P. A. Dayton, J. E. Chomas, A. L. Klibanov, and K. W. Ferrara, ''Experimental and theoretical evaluation of microbubble behavior: Effect of transmitted phase and bubble size,'' IEEE Trans. Ultrason. Ferroelectr. Freq. Control **47**, 1494–1509 (2000).
[39] R. E. Apfel, ''Possibility of microcavitation from diagnostic ultrasound,'' IEEE Trans. Ultrason. Ferroelectr. Freq. Control **33**, 139–142 (1986).
[40] C. K. Holland and R. E. Apfel, ''An improved theory for the prediction of microcavitation due to pulsed ultrasound,'' IEEE Trans. Ultrason. Ferroelectr. Freq. Control **36**, 204–208 (1989).
[41] W. D. O'Brien, Jr., D. G. Simpson, L. A. Frizzell, and J. F. Zachary, ''Superthreshold behavior and threshold estimates of ultrasound-induced lung hemorrhage in adult rats: Role of beamwidth,'' IEEE Trans. Ultrason. Ferroelectr. Freq. Control **48**, 1695–1705 (2001).
[42] K. Raum and W. D. O'Brien, Jr., ''Pulse-echo field distribution measurement technique of high-frequency ultrasound sources,'' IEEE Trans. Ultrason. Ferroelectr. Freq. Control **44**, 810–815 (1997).
[43] J. M. Sempsrott and W. D. O'Brien, Jr., ''Experimental Verification of Acoustic Saturation,'' in Proceedings of the 1999 IEEE Ultrasonics Symposium, 1999, pp. 1287–1290.
[44] J. M. Sempsrott, ''Experimental Evaluation of Acoustic Saturation,'' MS thesis, Department of Electrical and Computer Engineering, University of Illinois, Urbana, IL, 2000.
[45] *Acoustic Output Measurement Standard for Diagnostic Ultrasound Equipment* (American Institute of Ultrasound in Medicine, Laurel, MD, and National Electrical Manufacturers Association, Rosslyn, VA, 1998).
[46] *Standard for the Real-Time Display of Thermal and Mechanical Acoustic Output Indices on Diagnostic Ultrasound Equipment, Rev 1* (American Institute of Ultrasound in Medicine, Laurel, MD, and National Electrical Manufacturers Association, Rosslyn, VA, 1998).
[47] *Use of Mechanical Index in Place of Spatial Peak, Pulse Average Intensity in Determining Substantial Equivalence* (Center for Devices and Radiological Health, US Food and Drug Administration, Rockville, MD, 14 April 1994).
[48] *Information for Manufacturers Seeking Marketing Clearance of Diagnostic Ultrasound Systems and Transducers* (Center for Devices and Radiological Health, US Food and Drug Administration, Rockville, MD, 30 September 1997).
[49] G. A. Teotico, R. J. Miller, L. A. Frizzell, J. F. Zachary, and W. D. O'Brien, Jr., ''Attenuation coefficient estimates of mouse and rat chest wall,'' IEEE Trans. Ultrason. Ferroelectr. Freq. Control **48**, 593–601 (2001).