The Impact of Hemorrhagic Shock on Lung Ultrasound–Induced Pulmonary Capillary Hemorrhage

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Objectives—Lung ultrasound (LUS) exposure can induce pulmonary capillary hemorrhage (PCH), depending on biological and physical exposure parameters. This study was designed to investigate the variation in the LUS induction of PCH due to hemorrhagic shock, which itself can engender pulmonary injury.

Methods—Male rats were anesthetized with isoflurane in air. Shock was induced by withdrawal of 40% of the blood volume and assessed by the blood pressure detected by a femoral artery catheter and by blood glucose tests. B-mode ultrasound was delivered at 7.3 MHz with a low output (−20 dB) for aiming and with the maximal output (0 dB) for exposure. Pulmonary capillary hemorrhage was quantified by an assessment of comet tail artifacts in the LUS images and by measurement of PCH areas on the surface of fresh lung samples.

Results—Tests without shock or catheterization surgery gave results for PCH similar to those of previous studies using different methods. Exposure before hemorrhagic shock gave a mean PCH area ± SE of 24.8 ± 9.2 mm^2 on the ultrasound scan plane, whereas exposure after shock gave 0 PCH ($P < .001$; n = 7).

Conclusions—The presence of hemorrhagic shock significantly reduces the occurrence of LUS-induced PCH.

Key Words—bioeffects of ultrasound; B-lines; diagnostic ultrasound safety; lung ultrasound; ultrasound dosimetry

Lung ultrasound (LUS) has found valuable application to situations when a simple and rapid examination is needed for patient assessments in pediatrics, emergency medicine, intensive care, and point-of-care settings. Clinical LUS examinations are valuable in the diagnosis of pneumonia, pulmonary edema, pulmonary embolism, atelectasis, diffuse parenchymal disease, respiratory distress syndrome, and lung cancer.[1–3] Diagnostic ultrasound (US) does not penetrate well into normal lung, but the lung surface image can show signs of injury, edema, and interstitial lung disease. An important sign of lung disease is the detection of multiple reflection artifacts known as comet tail artifacts (CTAs, also known as B-lines), which extend inward from the bright reflection of the pleura.[2] For example, LUS assessments of CTAs and other features are valuable in emergency medicine for trauma.[4,5] The specific rapid US in shock (RUSH) examination is commonly applied to the critically ill for assessing pneumothorax, pulmonary edema and hemorrhage, and hydrothorax.[6] In risk–benefit considerations, US can avoid ionizing radiation and have an

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Abbreviations

ASK, after hemorrhagic shock; BSK, before hemorrhagic shock; CTA, comet tail artifact; LUS, lung ultrasound; PCH, pulmonary capillary hemorrhage; SI, shock index; UD, ultrasound delay; US, ultrasound

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exceptional safety profile for the patient. However, LUS presents the sonographer with a potential for local lung injury in some patients.[7,8] The use of LUS is widespread, particularly in point-of-care settings for monitoring patient status. Therefore, the specific use with shock is uncertain in relation to biological and physical dosimetric parameters associated with potential injury by exposure to LUS.

Pulmonary injury from diagnostic US takes the form of capillary hemorrhage into the alveoli.[7,9] In LUS imaging of rats, the US image displayed a CTA associated with the occurrence and progression of pulmonary capillary hemorrhage (PCH) with time.[10] Animal research has shown that PCH depends on the pulse amplitude, indicated by the on-screen mechanical index, and follows a threshold dose–response dependence. Additional physical parameters are also important, including the US frequency,[11] imaging duration,[12] and US mode.[13] An important feature of PCH is that biological factors also are very important, including sedation,[14] ventilation,[15] age, lung position,[16] and animal species.[17] The large possible range of patient physical US exposures and biological factors greatly complicates the assessment of possible clinical risk. The CTA displayed when diagnostic imaging induces PCH would not be useful for real-time safety guidance because the same sign is used for the diagnosis of patient conditions.[8] During an examination, not only could LUS conceivably display an ongoing CTA due to PCH induction and not be recognized as iatrogenic lung injury, which should be mitigated, but it also could confuse the interpretation of a CTA as diagnostic information.

Several factors common for emergency and clinical patient treatment have been investigated using our rat model of LUS. Fluid infusions commonly used for patient stabilization and resuscitation were tested as a perturbation of the blood side of the blood-air barrier. Pulmonary capillary hemorrhage was virtually eliminated in rats by rapid venous infusion of 35-mL/kg saline.[18] Intermittent positive pressure ventilation in anesthetized intubated rats, which perturbed the air side of the blood-air barrier, was also tested with exposure to diagnostic imaging.[15] When rather small end-expiratory pressures of ± 4 cm H_2O were applied, the PCH was virtually eliminated by positive pressure but strongly enhanced by negative pressure. The fraction of inspired oxygen also was investigated for its influence on LUS PCH induction using Telazol (Zoetis, Parsippany, NJ) anesthesia with and without xylazine.[19] Adding xylazine tends to enhance susceptibility to PCH. A reduced PCH effect with a 10% fraction of inspired oxygen was enhanced with the use of xylazine, whereas a 60% fraction of inspired oxygen enhanced the PCH effect especially for anesthesia with Telazol only.

These results show that interventions (fluid infusion ventilation and hyperoxia) associated with emergency patient management had important but varied influences on PCH induction by LUS. Other medical interventions or patient conditions might also yield important variations in LUS-induced PCH. Shock due to hemorrhage or trauma itself can engender pulmonary injury.[20,21] This tendency for shock-related lung injury led us to a hypothesis that animals in shock could have a greater susceptibility to US-induced PCH, which might worsen the impact of shock injury by adding LUS-induced PCH. Animal models are extensively used for clinically relevant studies of shock.[22] Isoflurane is the common anesthesia for the rat model of hemorrhagic shock[20,21,23] and has been shown to be useful in US PCH research.[24] This study was designed to investigate the variation of the diagnostic US induction of PCH due to the existence of hemorrhagic shock in isoflurane-anesthetized rats.

Materials and Methods

Animal Preparation

All in vivo animal procedures were conducted with the approval and guidance of the Institutional Animal Care and Use Committee of the University of Michigan. Male Sprague Dawley rats (Charles River, Wilmington, MA) were used for this research and maintained in the animal housing rooms of the Unit of Laboratory Animal Medicine. The rats weighed an average of 428 ± 36 g at the time of testing. Isoflurane in medical air was used to induce anesthesia (5%) and maintain the suitable level of anesthesia (0.5%–1.5%). The right thorax of each rat was shaved and depilated for US transmission.

The rats were mounted on a plastic holder fitted with a nose cone that was connected to the anesthesia machine (SurgiVet Isotec 4; Smiths Medical ASD,

Inc, St Paul, MN). The rats were placed in left lateral recumbency and instrumented with a heart rate and oxygen saturation sensor on a front paw (SurgiVet V3395 TPR; Smiths Medical Inc, St Paul). For exposure to US, the rat and mounting holder were placed horizontally on a warming pad to maintain body temperature, which was monitored by a rectal probe. Coupling of the US to the chest wall was accomplished with a shallow dish filled with warm water. A US transmission window in the bottom of the dish was coupled to the chest wall with US gel. This water standoff allowed consistent aiming and placement of the focal zone of the diagnostic US transducer at the lung surface for imaging exposure.

A femoral vein catheter was set for blood withdrawal. Hemorrhagic shock was produced by slowly withdrawing blood over 15 minutes, totaling an estimated 40% of the total blood volume. The total blood volume in milliliters was calculated by a standard formula for rats of 0.06 × body weight in grams +0.77 mL.[25] For example, 40% of the blood volume was estimated to be 10.5 mL for a 425-g rat.

A noninvasive blood pressure measurement system (Coda monitor; Kent Scientific Corp, Torrington, CT) was used on the tail of some rats to noninvasively measure blood pressure. The system involved placement of an occlusive cuff and a sensing cuff on the rat tail. The blood pressure signals were sent to a personal computer via a universal serial bus port, recorded, and analyzed with the aid of the Coda software. The system automatically made a series of blood pressure determinations, which stabilized after several readings. The 10th reading was chosen as the optimal determination and used for this study for readings at initial, intermediate, and final time points. This system was initially considered for use as the primary measurement method during shock to avoid the trauma and procedure delay of femoral artery catheterization. However, the results were not valid for blood pressure values much less than normal and could not be used for the low blood pressures produced by the hemorrhage procedure.

Blood pressure measurements for the tests of hemorrhagic shock were made with a blood pressure catheter (SPR-320, size 2F, Millar, Inc, Houston TX). The catheter was inserted into a femoral artery and the sensor advanced to the abdominal aorta. This surgical procedure was impactful and often involved a lengthy period of time, lasting up to 60 minutes, in the handling of the rats for the experiment. The blood pressure signal was amplified with a bridge amplifier and calibrated with a calibration kit (FE221 and MLA1052; ADInstruments, Inc, Colorado Springs, CO). The signals were digitized (Powerlab 4/30; ADInstruments, Inc), recorded, and analyzed with the aid of software (Chart Pro 5, version 5.5.5; ADInstruments, Inc). Systolic and diastolic blood pressures were recorded. A shock index (SI) was calculated as heart rate/systolic blood pressure.[23,26] This index was indicative of the shock severity, with increases to greater than 200% suggestive of a lethal hemorrhage.[23]

In addition, blood glucose was measured as a general indicator of trauma and blood loss,[27,28] which has proven useful in shock research in rats.[29,30] A blood glucose meter (Contour 7151H; Bayer HealthCare, Berlin, Germany) was used for measurements using the standard human Contour test strips with a drop of blood. The blood glucose tends to rise after hemorrhagic shock and the ratio of blood glucose before and after shock was also used as a shock indicator.

Ultrasound

The diagnostic US machine used for imaging and exposure was a clinical Vivid 7 Dimension system (GE Vingmed Ultrasound AS, Horten, Norway) with a 10 L linear array transducer. The transducer was held by a rigid gantry in fixed position above the rat and aimed between ribs by using a gimbal mount. B-mode imaging was used with a 3-cm image depth and a 1.3-cm single focus, which was placed at the lung surface. Parameters of the US pulses were measured at the position of the maximum (1.3-cm depth on the transducer axis) peak rarefactional pressure amplitude by using a calibrated hydrophone with a 0.2-mm sensitive spot (HGL-0200; Onda Corp, Sunnyvale, CA) and are listed in Table 1. To approximate in situ values at the lung surface, the digitized pulse pressure waveform, measured in water, was derated by an attenuation factor of 1.2 dB/cm-MHz,[13] which was −6.0 dB for the mean chest wall thickness of 0.68 ± 0.07 cm. The measured US frequency was 7.3 MHz, with 60.2 frames per second, a 96-microsecond pulse repetition period, and a 270-nanosecond pulse duration. The −6-dB width of

Table 1. Ultrasound Exposure Parameters Obtained With the 2 Exposure Power Settings for the Observed US Frequency of 7.3 MHz and Derated (−6 dB) for Attenuation In Situ

Setting, dB	MI_{OS}, MPa $MHz^{-½}$	PRPA, MPa	PMPA, MPa	MI_{IS}, MPa $MHz^{-½}$	I_{SPPA}, W cm^{-2}
0	1.2	2.6	4.2	0.95	307
–20	0.12	0.31	0.33	0.11	2.8

I_{SPPA} indicates spatial-peak pulse-average intensity; MI_{IS}, mechanical index calculated in situ; MI_{OS}, mechanical index presented on screen; PMPA, pulse mean pressure amplitude; and PRPA, peak rarefactional pressure amplitude.

the beam was 1.2 mm. A − 20-dB power setting (pressure amplitudes reduced by a factor of ≈10) was used for aiming and recording images before and after US images. For exposure, the power was quickly raised to the maximum setting (0 dB) and timed for 5 minutes, after which the power was quickly lowered again to −20 dB.

The transducer was carefully aligned between two ribs to give a bright image of the lung surface, seen as a curved line across the image (Figure 1). The image indicated approximately perpendicular incidence by presentation of a multiple reflection artifact (A-line) at about 2.5 cm in the 3-cm-deep image. During and after US exposure, the images provided information about the PCH effect by displaying CTAs projecting inward perpendicular to the lung surface image. This artifact gives a qualitative real-time indication of PCH induced by the US exposure. The width of the bright-line lung surface in the image was measured, together with the width of the line that was involved with the CTA. The percentage width of the CTA across the bright-line image was calculated as one measure of the PCH effect.

At the end of the tests, each rat was euthanized under anesthesia by exsanguination of the inferior vena cava, as described previously.[15] The trachea was tied off and the lungs and heart removed intact. Photomicrographs of the affected right cranial and medial lobes were used to measure the approximate area of the PCH regions on the lung surface (Spot, version 5.1, Diagnostic Instruments, Inc, Sterling Heights, MI), which was used as the quantification of the overall PCH impact.

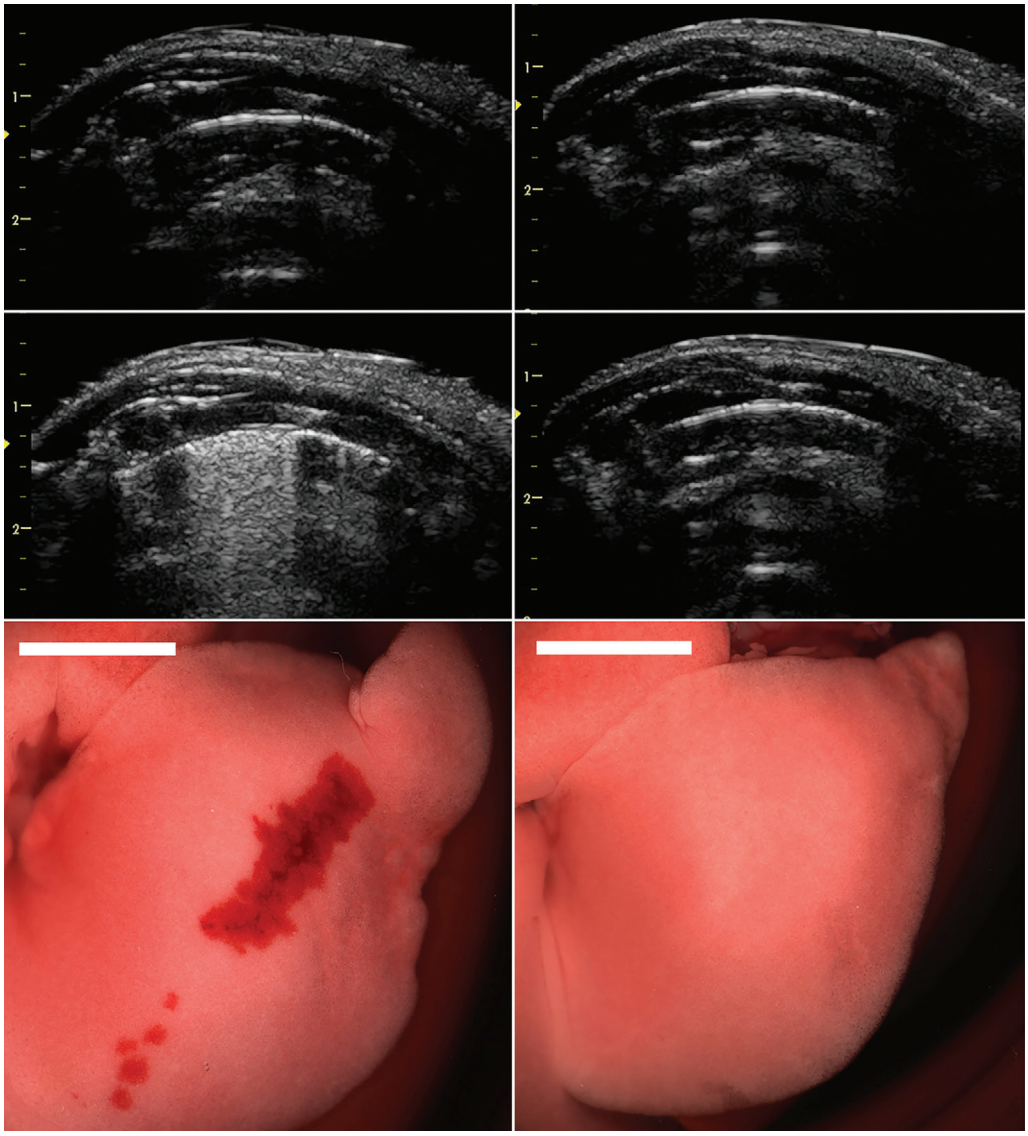
Experimental Plan and Statistics

For this study, a total of 35 male rats were randomly assigned to 5 groups of 7 rats each. The key test, to determine the impact of hemorrhagic shock on LUS-induced PCH, was to perform US exposure either before hemorrhagic shock (BSK) or after hemorrhagic shock (ASK). Initial planning had included groups to build data for an exposure-response PCH threshold determination for the BSK and ASK tests, but the results were so definitive (see "Results") that only the 0-dB exposure was used. The methods used in this study differed substantially from our previous studies.[11,24] Previous work had used female rats and vertical positioning of the rats for exposure in a water bath and did not require lengthy surgery for arterial catheter placement. Three groups of rats were used for gauging the possible changes in PCH results due to the different methods used for this study. A sham procedure and US administration with 30-minute (UD30) and 60-minute (UD60) delays in the procedure were performed with male rats and a horizontal position but without surgery or shock for comparison to previous measurements. The statistical analysis was performed with SigmaPlot version 14.0 software for Windows (Systat Software, Inc, San Jose CA). The Student t test was used for comparisons between groups as a 2-tailed test (ie, including the possibility of a reduction in the PCH effect as well as the hypothesized increase) with statistical significance assumed at $P < .05$.

Results

The results for the physiologic parameters are listed in Table 2. The heart rate, oxygen saturation, and blood pressure were somewhat suppressed in all rats, presumably owing to the anesthesia protocol. For BSK and ASK groups, the US exposure time corresponded to the intermediate time point. The shock produced by the blood withdrawal was evidenced by

Figure 1. Examples of the LUS before (top) and after (bottom) exposure and the PCH seen on the lungs for BSK (left) and ASK (right). The CTA showing in the images faithfully indicated the distribution of the PCH occurrence along the line corresponding to the US scan plane on the right medial lobes.



the statistically significant reduction in blood pressure for BSK (end relative to the intermediate time point) and for ASK (intermediate relative to the start time point). In addition, the blood glucose level after the blood withdrawal was increased for ASK ($P < .05$) but not significantly so for BSK ($P = .09$).

Before and after LUS images of 1 BSK-exposed rat and 1 ASK-exposed rat are shown in Figure 1. The normal images from aiming (−20 dB) before the power was raised are the top images. With the LUS looking downward, the water standoff is seen as a black layer above the skin surface, which appears as a curved white line. The skin layer has a granular appearance, and horizontally, the sternum is shown to the left. The intercostal space, which also has some indications of nearby ribs, extends downward to the pleurae and lung gas surface, which is displayed as a bright white curved line at about a 1.3-cm depth. All

Table 2. Physiologic Parameters at the Initial, Intermediate (BSK and ASK Exposure) and Final Time Points

Group	Time Point	HR, Beats/min	Spo_2, %	SBP, mm Hg	DBP, mm Hg	Glucose, mg/dL
Sham	Start	326 ± 39	87 ± 4	86 ± 6	57 ± 9	196 ± 35
Sham	End	355 ± 49	90 ± 5	82 ± 9	61 ± 8	187 ± 27
UD30	Start	342 ± 21	91 ± 3	75 ± 16	59 ± 9	228 ± 40
UD30	End	359 ± 22	90 ± 5	75 ± 12	58 ± 6	225 ± 62
UD60	Start	357 ± 27	87 ± 4	96 ± 26	73 ± 19	194 ± 73
UD60	End	358 ± 39	87 ± 8	88 ± 16	65 ± 12	215 ± 87
BSK	Start	323 ± 22	89 ± 7	105 ± 20	70 ± 12	191 ± 37
BSK	Intermediate	307 ± 28	84 ± 7	103 ± 17	68 ± 15	204 ± 41
BSK	End	292 ± 51	79 ± 9	42 ± 6[a]	24 ± 6[a]	250 ± 59
ASK	Start	320 ± 24	88 ± 7	81 ± 15	56 ± 9	207 ± 37
ASK	Intermediate	295 ± 127	82 ± 8	53 ± 12[b]	33 ± 7[a]	293 ± 105[b]
ASK	End	293 ± 71	87 ± 8	69 ± 15	37 ± 12	291 ± 98

Data are presented as mean ± SD (n = 7). Hemorrhagic shock was seen as a significant decrease in systolic and diastolic blood pressure (BSK and ASK) and an increase in blood glucose (ASK). DBP indicates diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; and SpO2, oxygen saturation.
[a]Statistically significant difference ($P < .001$).
[b]Statistically significant difference ($P < .05$).

echoes shown below this bright line present image artifacts because the lung surface has mirrorlike acoustic reflectivity. The strong reflection from the lung generates a second lung surface image artifact (an A-line) at about a 2.6-cm depth due to a reflection from the surface of the transducer and a secondary reflection from the lung surface received by the transducer and displayed as the deeper image. The A-line indicates a clear image path and good alignment of the transducer for exposure. If PCH starts, a perturbation of the bright-line surface image begins to be discernable and gradually grows during the exposure to form the CTA, which appears to grow downward from the lung air surface. The final BSK image shows an extensive CTA distribution penetrating downward to the bottom of the image in some of the width of the bright-line lung image. The final ASK image shows absolutely no indication of a CTA. The photographs of the freshly removed lung samples (bottom) show the PCH effect as an irregular blood-red pattern. For BSK, the PCH extends along a line set by the US scan plane and tracks the CTA distribution. For ASK, there was no discernable indication of PCH on the lungs, again reported by the CTA observation by its absence.

The shock indicators and measured results for the PCH areas are presented in Table 3. The shock indicators of the SI and blood glucose ratio were useful and confirmed that shock had resulted from the hemorrhage. The SI strongly indicated shock for the BSK group, with the blood glucose ratio only giving a weak indication. For the ASK group, the SI increase was not statistically significant, but the blood glucose ratio increase was a statistically significant indication of shock. As noted in "Materials and Methods," the groups designated sham, UD30, and UD60 were included to assess the possible differences in the PCH effect from previous work, which might help interpret the BSK versus ASK results. In particular, a possible strong reduction in the PCH was postulated to occur due the relatively long delay after the start of anesthesia before the US exposure. However, the UD30 and UD60 exposures produced the expected PCH effect ($P < .001$ relative to sham), and there was no statistically significant difference between the UD30, UD60, and BSK results. The ASK result was the same as the sham result and was highly significantly different from the BSK result ($P < .001$). The PCH results are graphically displayed in Figure 2.

Discussion

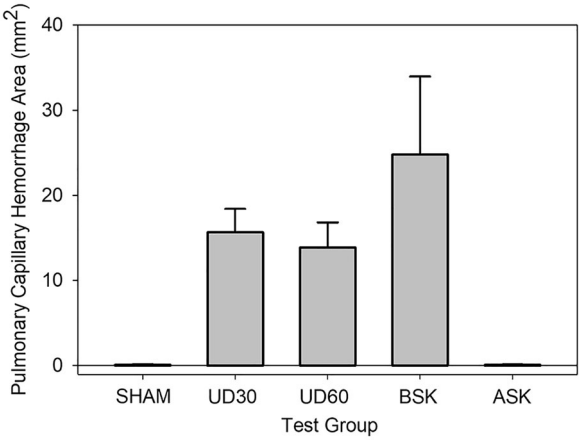
These results clearly show that our initial hypothesis, that hemorrhagic shock might enhance susceptibility

Table 3. Results for the Shock Parameters for LUS Exposures and the Measurements of the Resulting CTA and PCH

Group	SI, Start	SI, Intermediate	SI, End	BG Ratio, Intermediate/Start	BG Ratio, End/Intermediate	CTA, %	PCH, mm^2
Sham	4.3 ± 0.2	NA	4.3 ± 0.2	NA	0.98 ± 0.07	0	0
UD30	4.7 ± 0.3	NA	4.8 ± 0.4	NA	1.00 ± 0.08	55.6 ± 6.5[a]	15.7 ± 2.7[a]
UD60	3.9 ± 0.3	NA	4.2 ± 0.2	NA	1.13 ± 0.11	47.8 ± 6.5[a]	13.9 ± 3.0[a]
BSK	3.2 ± 0.2	3.0 ± 0.1	7.0 ± 0.3[b]	1.09 ± 0.09	1.24 ± 0.12	47.2 ± 7.5[a]	24.8 ± 9.2[a]
ASK	4.0 ± 0.3	5.6 ± 0.7	4.3 ± 0.4	1.41 ± 0.15[b]	1.00 ± 0.04	0	0

Data are presented as mean ± SE (n = 7). BG indicates blood glucose; and NA, not applicable.
[a]Statistical significance was shown for exposure versus sham or ASK.
[b]Statistical significance was shown for hemorrhage SI for BSK and blood glucose for ASK.

Figure 2. Results for PCH areas (mean and standard error) measured on the fresh lung samples for the maximal 0-dB exposure in groups of 7 rats. The PCH results for UD30, UD60, and BSK were highly significantly different from the sham and ASK results, which were both 0.



to the PCH bioeffect of LUS, was incorrect. The withdrawal of blood leading to shock completely eliminated the PCH effect obtained by the maximal exposure from our exposure system without shock. Apparently, the low blood pressure minimizes the tendency for capillary hemorrhage, certainly a plausible result. No further groups (eg, a dose response or graded variation of blood withdrawal) were evaluated because of the variability of the observed shock level in our tests and the perfectly clear result at 0 dB of a strong PCH effect for US BSK and 0 for US ASK.

The PCH results here seemed somewhat less than the effect for female rats with ketamine plus xylazine anesthesia using the Vivid 7 machine with the 10 L transducer.[13] However, the isoflurane anesthesia method was previously shown to yield a lesser PCH effect,[24] similar to the result found here. In addition, the use of male rats, which have a thicker chest wall of 6.8 mm compared to 5 mm for female rats, would be expected to give somewhat lower results because of the increased attenuation (−6 dB compared to −4.4 dB for the previous study with female rats). The horizontal position with standoff for these methods was somewhat more difficult to use in terms of aiming than the vertical mounting for the water bath setup used previously but again appears to give similar results. Overall, this study gave results comparable to those of earlier work, and those other methods would likely produce the same positive BSK and negative ASK results shown here for hemorrhagic shock.

The conclusion from this study is that shock does not enhance susceptibility to LUS-induced PCH, and in fact, hemorrhagic shock appeared to eliminate the effect obtained without shock. Examinations such as the rapid US in shock protocol should not involve consideration of an increased patient risk of PCH. This finding, together with the observation that saline infusion also seems to mitigate the PCH bioeffect,[18] indicates that an intervention on the blood side of the blood-air barrier induces physiologic changes that are less conducive to PCH induction. The interventions on the air side of the blood-air barrier by ventilation or hypoxia, in contrast, readily gave increased as well as decreased changes in observed PCH. Possibly, blood-side perturbation giving higher blood pressure, such as hypertension, may yield enhanced PCH susceptibility. A study using spontaneously hypertensive rats is in progress to test this hypothesis.

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