This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of record. Please cite this article as doi:10.1002/jum.14555.

Does IV Infusion Influence Diagnostic Ultrasound-Induced Pulmonary Capillary Hemorrhage?

Douglas L. Miller[1], PhD, Zhihong Dong[1], MD, Chunyan Dou[1], MD, Krishnan Raghavendran[2], MD.

Department of Radiology[1] and Department of Surgery[2]

University of Michigan, Ann Arbor, MI. 48109

Corresponding author: Douglas L. Miller, PhD, FAIUM

3240A Medical Sciences Building I

University of Michigan Health System

1301 Catherine Street

Ann Arbor MI 48109-5667

Tel: (734) 647-3344

FAX: (734) 764-8541

Email: douglm@umich.edu

Abstract

Objectives: Pulmonary diagnostic ultrasound (PDUS) can induce pulmonary capillary hemorrhage (PCH) in mammals. This singular biological effect of diagnostic ultrasound imaging was discovered over 25 years ago, but remains poorly understood. Our objective here was to investigate rapid infusion of IV fluids as a possible stressor for capillaries, which might enhance PDUS-PCH.

Methods: Rats were anesthetized with Telazol, which yielded relatively low PDUS-PCH, or Telazol and xylazine, which yielded relatively high PDUS-PCH. Groups of rats were not infused or infused either with normal saline, 10% mannitol or 5% albumin. Rats were scanned in a warmed water bath with B mode ultrasound for 5 min with a 7.6 MHz linear array set to different Mechanical Index values to obtain exposure response information. PCH was observed as comet tail artifacts in the image, and measured on the lung surface.

Results: For Telazol anesthesia, all the PCH results were very low, with no significant differences at the maximum output with an in situ peak rarefactional pressure amplitude of 2.1 MPa (on screen Mechanical Index, MI_{os}=0.9). The addition of xylazine to the Telazol anesthetic significantly enhanced the PCH ($P<0.001$) without infusion and likewise for the mannitol and albumin infusion. Saline infusion eliminated this enhancement, with significantly reduced PCH for Telazol plus xylazine anesthesia ($p<0.001$); however, both mannitol and albumin infusion resulted in significantly more PCH than saline infusion ($p<0.01$).

Conclusions: These results show PCH dependence on the specific IV infusion fluid, and illustrate the complex importance of physiological parameters for ultrasound-

induced PCH.

Key words: Pulmonary ultrasound, Mechanical index, Comet-tail artifact, Crystalloid and colloid infusion; Diagnostic ultrasound safety

Introduction

Diagnostic ultrasound imaging of mammalian lung can induce pulmonary capillary hemorrhage (PCH). The induction of PCH by pulsed ultrasound was discovered more than 25 years ago [1], but remains poorly understood. Reviews of research have indicated that the phenomenon occurs in mice, rats and pigs and may be characterized by a threshold for a specific situation [2, 3]. Initially, the lung was expected to receive mostly incidental exposure, such as during echocardiography, and therefore no specific guidance was devised for avoidance of the effect [3]. At this time, however, direct pulmonary diagnostic ultrasound (PDUS) has become routine in intensive care, emergency care and point-of-care medical settings [4-7]. PDUS has been found to be valuable in diagnosis of pneumonia, pulmonary edema, embolism, pneumothorax, atelectasis, diffuse parenchymal disease, respiratory distress syndrome, and lung cancer [8]. The use of portable ultrasound machines allows PDUS to be performed by the physician at the bedside, essentially replacing the stethoscope [9-11]. This rapidly expanding use of PDUS lends some urgency to efforts to define the possible risks of PCH for patients and to provide suitable safety guidance.

Our research has used actual diagnostic ultrasound systems (early work utilized single element laboratory systems) to provide relevant information including the PDUS images which display the occurrence of PCH as comet tail artifacts extending from the pleura toward the interior [12]. The thresholds for this bioeffect appear to have little frequency dependence [13], which suggests that the mechanism of acoustical radiation pressure may play a role in the induction of PCH [14]. PCH has a strong dependence on poorly defined physiological conditions: the anesthesia methods, such as use of

xylazine, are very important for PDUS induced PCH in rats [15]. The enhancement phenomenon also occurs for dexmedetomidine, a common clinical sedative that causes little or no respiratory depression [16]. These findings imply that many patient medical treatments could influence PDUS-PCH in uncertain ways. Some patient conditions or treatments may worsen the risk while others may alleviate the risk.

The finding that the PCH effect is low for ketamine, and higher for ketamine plus xylazine anesthesia [15] provided a means to test drug treatments or other physiological conditions for their influence on the bioeffect. For ketamine, conditions leading to enhanced PCH may be more evident than for ketamine plus xylazine, which already enhances the PCH. Conversely, conditions leading to reduced PCH may be more evident for ketamine plus xylazine than for ketamine anesthesia, which already has a low effect. Therefore, testing rat models of various clinical conditions both with ketamine and ketamine plus xylazine anesthesia could readily identify conditions which either enhance or reduce PCH. One problem with this duel strategy is that ketamine, a dissociative agent [17], provides poor muscle tone, immobility and analgesia at normal doses. Telazol, which consists of tiletamine (related to ketamine) plus zolazepam (benzodiazepine-related tranquilizer), is an attractive alternative to ketamine, and provides light anesthesia with immobility. In this study, Telazol was compared to Telazol plus xylazine for use as the dual strategy of evaluating physiological conditions for their influence on PDUS-PCH.

A conceptually simple physiological condition is the infusion of IV fluids. Rapid infusion of IV fluids, such as saline, can lead to hypervolemia and eventual pulmonary edema identifiable by diagnostic ultrasound B-lines [18]. Hypothetically, fluid infusion

leading to vascular hypervolemia and possibly to pulmonary edema may do so by stressing pulmonary capillaries, thus predisposing the lung to PCH. Saline is a crystalloid infusion fluid which rapidly distributes between vascular and tissue interstitium compartments. Another clinical IV fluid is mannitol, a sugar alcohol used at hypertonic concentration to de-hydrate tissues, such as to reduce intracranial pressure due to severe traumatic brain injury owing to its inability to cross the blood brain barrier. Mannitol also can cause diuresis possibly reducing edema, such as pulmonary edema [19]. Clinical IV infusion of albumin solution can produce increased pulmonary blood volume without potentially increasing pulmonary edema [18]. IV albumin is a colloid fluid which tends to remain within the vasculature, thus increasing plasma volume. Rapid infusion of very high volumes (e. g. 10% of body weight) of saline, or mannitol or plasma, can lead to life threatening fluid overload, which tends to produce pulmonary interstitial edema for saline, alveolar edema for plasma (albumin) and both for hypertonic mannitol [20]. In the current study, PDUS-PCH variation from infusion of these fluids was assessed using the strategy of Telazol in comparison to Telazol plus xylazine anesthesia.

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 (IACUC) of the University of Michigan. Female rats (Sprague Dawley, Charles River, Wilmington, MA, USA) were used for this study, as described previously [12]. Anesthesia was accomplished either with Telazol

(Zoetis Inc., Kalamazoo, MI USA) 90 mg/kg only (TO) or with Telazol and xylazine (Xylamed injection, Bimeda-MTC Animal Health Inc. Cambridge ON CA) 9 mg kg^{-1} (TX). The use of xylazine for anesthesia was previously shown to enhance PDUS-PCH [15], possibly due to an impact on pulmonary capillaries. The use of high doses of xylazine is also associated with induction of pulmonary edema [21, 22]. The right thorax of all rats was shaved and depilated for ultrasound transmission. The rats were mounted in a 38 °C degassed water bath for ultrasound exposures of the right lung. This water bath method provides reproducible ultrasound coupling and exposure, and maintains the body temperature of the rats.

A jugular vein catheter was set for IV injection of fluids in the second part of the study. Three IV fluids were used: Normal saline (0.9 % sodium chloride injection, Hospira.com) was the standard medical IV solution administered for fluid replenishment. Mannitol 10 % (Osmitrol 10 % Mannitol Injection, Baxter Healthcare.com) was a hypertonic diuretic which acts to dehydrate tissue with 596 mOsmol. Albumin (Albumin 5% Solution, CSL Behring LLC, Kankakee, IL USA) was given as a volume expanding colloid with normal osmolarity (310 mOsmol). These fluids were injected rapidly using a syringe pump at 7 ml/kg/min for 5 min just before ultrasound scanning, which was intended to perturb the pulmonary capillaries in these acute tests. The rats were then scanned by diagnostic ultrasound for 5 min followed by euthanasia 5 min later. This infusion volume totaled 3.5% of body weight (about 50% of blood volume). This level of infusion did not produce discernable stress in the rats over the time period of the tests, which can occur for higher infusion volumes. In the acute study of Manenti et al. [20], infusion of 10% of body weight of these IV fluids resulted in tachypnea and cyanosis,

followed by death within 15 min.

Ultrasound

A Phillips HDI 5000 (Philips Healthcare, Andover MA USA) diagnostic ultrasound machine with CL15-7 linear array was used for B mode scanning with 2 cm image depth, 1 cm focal depth, and 39 frames per second, as described previously [12]. The probe was set up in the water bath using an adjustable gantry to aim through an intercostal space at the right cranial or medial lobe of the rat lung. The pleural surface was at a depth of about 5-6 mm, with the probe partially in contact with the skin. The pulses of ultrasound had a center frequency of 7.6 MHz with a pulse repetition frequency of 10 kHz. The on-screen Mechanical Index (MI_{os}) was set to 0.21 for aiming, and then quickly raised to MI_{os} = 0.37, 0.52, 0.7 or 0.9 (maximum for this probe) for 5 min of scanning. The acoustical parameters were measured using a calibrated hydrophone as described previously [13], and are listed in Table 1, including peak rarefactional pressure amplitude (PRPA), peak mean pressure amplitude (PMPA), and spatial peak pulse average intensity (I_{SPPA}).

Measured endpoints

Measured physiological parameters included heart rate and SpO_2 (Physiosuite, Kent Scientific Corp., Torrington, CT USA). This system allowed measurement of higher heart rates than possible with standard veterinary monitors. The wet/dry weight of the left lobe of the lung was measured by weighing the sample, drying for 24 hours at 37 °C, and re-weighing. For the rats with infusion, the hematocrit was measured in baseline samples and in post-test samples. This information was used to estimate the percentage increase in blood volume accomplished by the infusions as 100 times the

difference between the post-test hematocrit and baseline, divided by the baseline result. In addition, a non-invasive blood pressure system (Coda Monitor, Kent Scientific Corp., Torrington, CT USA) was used on the tail of the rats to measure blood pressure before and after the test.

Exposure related endpoints included the percentage of the bright-line image of the lung surface, which was involved with comet tail artifacts (CTAs) at the end of exposure, and measurement of PCH areas on the lung surface, as described previously [12,13, 15, 16]. Briefly, upon euthanasia, the trachea was tied off, and the lungs were removed. The right lobes, which were the target of the imaging, were then examined and photographed using a stereomicroscope with digital camera (Spot Flex, Diagnostic Instruments Inc., Sterling Heights, MI USA). The photographs of the lungs were used to measure the approximate diameter and area of the region of PCH on the lung surface using image analysis software (Spot v. 5.1, Diagnostic Instruments, Inc., Sterling Heights, MI USA). The PCH was readily identified as irregular bright red areas, along the line of the ultrasound scan plane and often the same length as the CTA length in the image. These were manually outlined using the software with the appropriate scale calibration, which gave the area in mm^2.

Experimental plan and statistics

The comparison of Telazol only (TO) to Telazol plus xylazine (TX) anesthesia was performed first, and the results indicated that the different anesthetics provided a reasonable dual test strategy. This finding led to performance of the experiments with TO and TX for each infusion solution. The two parts were done using the same methods, except for the added hematocrit and blood pressure measurements for the

infusion part. Physiological parameters for the experimental conditions are listed in Table 2. The addition of xylazine for TX anesthesia gave significant reductions in heart rate, SpO_2, and blood pressure relative to TO, as expected. For each condition, groups of 5-6 rats were scanned at the maximum MI_{os} value of 0.9, and additional lower values including MI_{os} = 0.7, 0.52 and 0.37. For reference, the guideline upper limit for diagnostic ultrasound is MI_{os}=1.9, substantially higher than available from the small, high resolution probe. The response for TO conditions was very low for the infusion tests, and only MI_{os}=0.9 groups were included. One sham TX group was included using the MI_{os} for aiming only. A total of 42 rats (7 groups of 6) were included in the TO versus TX test, and 65 rats were included in the infusion experiment (13 groups of 5). The groups and exposure parameters are presented in Table 3. Statistical comparisons used the Z test of proportions for comparison of two groups, with significance assumed at $p \leq 0.053$ ($p=0.053$ corresponds to 4/5 positive results, compared to 0/5 for shams). The results were unexpectedly mostly negative. Therefore, the lowest MI_{os} groups for each TX condition, which was extended to lower values until no positive effect found, was considered to be a sham. Two way analysis of variance (ANOVA) analyses were conducted to compare the importance of the MI_{os} exposure setting relative to the addition of xylazine (TO versus TX) and relative to the infusion solutions (saline, mannitol and albumin). In addition, linear regression was performed on the exposure response trends of groups including any PCH detected, to estimate thresholds as the zero crossing point.

Results

The general physiological measurements are listed in Table 2. The differences in heart rate (HR), oxygen saturation (SpO_2), and blood pressure were all highly

significantly different ($p<0.01$) between Telazol only and Telazol plus xylazine anesthesia. The MBP values were variable using the non-invasive method, and there were no significant differences between before (bef) and after (aft) values. For Telazol wet dry ratios, there was a significant decrease for saline infusion, no significant difference for mannitol infusion but a significant increase for albumin infusion. For Telazol plus xylazine, all the wet/dry results for the infusions were significantly greater than for no infusion. The hematocrit of about 0.45 was reduced for all three infusions by 18-27 % with a statistically significant blood volume increase for albumin infusion relative to no infusion.

The measured endpoint results are presented in Table 3. The primary measure of the effect was the PCH area. The comet tail artifacts were also measured and closely tracked the length PCH on the lungs. For the comparison of TO and TX anesthesia, the added xylazine yielded a significant increase in PCH at MI_{os}=0.9 ($p<0.001$), and more observed PCH at lower MI_{os} values. The ANOVA showed that the xylazine had a significant impact ($p<0.001$) as did the MI_{os} setting ($p<0.001$); furthermore, the MIos = 0.9 setting was statistically significantly different from MI_{os} =0.37, 0.52 and 0.7. This observation was similar to ketamine compared to ketamine plus xylazine results [15], and indicated that the strategy of TO versus TX tests for gauging the influence of different conditions on PCH sensitivity was plausible.

Comparison between the no-infusion and infusion parts of the study revealed no substantial enhancement due to the infusions, as shown in Fig. 1 for the MI_{os}=0.9 setting. All the infusions for TO led to PCH, which was not significantly different from zero, apparently reducing the significant PCH seen for TO without infusion. All of the PCH

results with TX were significantly greater ($p<0.01$) than the corresponding TO condition except for the saline infusion which was somewhat less ($p=0.078$). However, the saline infusion with TX produced a substantial reduction in PCH relative to the TX without infusion ($p<0.001$).

For the infusion part of the study, no significant PCH was observed for TO anesthesia (Figure 1). For TX anesthesia, the PCH for mannitol or albumin infusion were comparable to the no-infusion result (Fig. 1). The PCH for saline infusion was very low, and not significantly different from zero even for TX. The ANOVA analysis indicated that both mannitol and albumin infusion with TX gave relatively large PDUS-PCH, which were significantly greater than the result for saline infusion ($p<0.005$ for mannitol and $p<0.001$ for albumin). Furthermore, both the MI_{os} =0.9 ($p<0.001$), and 0.7 ($p=0.015$) results were significantly different from MI_{os} =0.37 (taken as the sham).

Threshold determination by linear regression provides an important measure of sensitivity, which utilizes all the data points. For the infusion TO conditions with only one point (MI_{os}=0.9, Table 3), the threshold could not be determined by linear regression, but may be assumed to be approximately equal to the maximum exposure PRPA = 2.1 MPa. The data sets for determinations of thresholds with TX were small, as shown in Figure 2, and the linear regressions had modest coefficients of determination (r^2)(Table 3). For the TX without infusion and with mannitol and albumin infusions, the zero intercepts for linear regression were all about the same, ranging from PRPAs of 1.2 to 1.4 MPa, see Table 3. For TO with no infusion and TX with saline infusion, the zero crossings were 1.6-1.7 MPa, about one exposure step higher than those for the other conditions.

Discussion and Conclusion

One goal of this study was to compare PDUS-PCH results obtained with TO and TX anesthesia as a means to evaluate enhancement or reduction of the PCH bioeffect by physiological conditions. The PCH effect appeared to be somewhat inhibited relative to previous observations with this ultrasound machine and probe using ketamine [12], rather than Telazol. The MI_{os}= 0.52 yielded statistically significant PCH for KX (ketamine and xylazine) anesthesia in that study, but not in this study with TX anesthesia. However, the TO condition produced very similar results to KO (ketamine) in a previous study [15]. The Telazol mixture provides much better immobilization than ketamine only, and analgesia sufficient for minor surgical procedures, such as placement of the jugular vein catheter.

The TO and TX comparison was used to test for PCH reduction or enhancement by IV fluid infusion. The main finding in this study was that infusion tended to reduce PCH. The TO anesthesia without infusion gave statistically significant PCH ($p<0.02$) at MI_{os}=0.9, but TO conditions with infusion produced no significant PCH. An overall comparison of the 5/6 proportion of occurrence for TO without infusion with 3/15 occurrence for TO with any infusion was a significant reduction at $p=0.028$. For TX anesthesia, saline infusion eliminated the enhancement by xylazine. The mannitol and albumin infusions had no clear impact on the PCH relative to the no-infusion part of the study. However, within the infusion part of the study, the comparison of saline to albumin infusion was striking: Saline infusion with TX did not give statistically significant PCH at MI_{os}=0.9. However, mannitol and albumin infusion resulted in observed PCH, which was significantly greater ($p<0.001$) than for saline infusion.

In conclusion, the TO and TX strategy for assessing conditions favoring or

inhibiting PDUS PCH was validated, with lower PCH for TO and higher for TX. For infusion, the most substantial change was the elimination of significant PDUS PCH for saline infusion with TX and the reduction to insignificance of PCH by all the infusions for TO anesthesia. The mechanism for the reduction in the PCH effect by saline infusion is uncertain. Because it is neutral with regard to tonicity and subject to redistribution outside the vasculature, the rapid infusion of this crystalloid gave the lowest increase in blood volume. The striking difference between the results for saline infusion and albumin infusion ($p<0.001$) probably arose from the different disposition of crystalloid and colloid infusions. Albumin and mannitol have a much greater tendency than saline to remain within the vasculature, increasing plasma volume and potentially provoking distension and stress, as well as recruitment, of pulmonary capillaries, which could increase vulnerability to PDUS-PCH. Using the TO and TX test strategy, the infusion did not increase PCH for TO but rather decreased it. For TX, albumin and mannitol had little effect, but saline reduced PDUS-PCH to a level even lower that the TO result. Overall, the hypothesis that rapid infusion of IV fluids could serve as a possible stressor for pulmonary capillaries, which might enhance PDUS-PCH, was not supported.

Acknowledgments:

This study was supported by the National Heart Lung and Blood Institute via grant number HL116434. The information contained herein does not necessarily reflect the position or policy of the US government, and no official endorsement should be inferred.

References:

1. Child SZ, Hart man CL, Schery LA, Carstensen EL. Lung damage from exposure to pulsed ultrasound. Ultrasound Med Biol. 1990;16:817-25.
2. American Institute of Ultrasound in Medicine [no authors listed]. Section 4--bioeffects in tissues with gas bodies. J Ultrasound Med. 2000;19:97-108, 154-68.
3. Church CC, Carstensen EL, Nyborg WL, Carson PL, Frizzell LA, Bailey MR. The risk of exposure to diagnostic ultrasound in postnatal subjects: nonthermal mechanisms. J Ultrasound Med. 2008;27:565-92.
4. Volpicelli G. Lung sonography. J Ultrasound Med. 2013;32:165-171.
5. Lichtenstein D. Lung ultrasound in the critically ill. Curr Opin Crit Care. 2014;20:315-322.
6. Ahmad S, Eisen LA. Lung ultrasound: the basics. In: Lumb P and Karakitsos D eds. Critical care ultrasound. Philadelphia: Elsevier, 2015. Ch 19, pp 106-110.
7. Dietrich CF, Goudie A, Chiorean L, Cui XW, Gilja OH, Dong Y, Abramowicz JS, Vinayak S, Westerway SC, Nolsøe CP, Chou YH, Blaivas M. Point of care ultrasound: a WFUMB position paper. Ultrasound Med Biol. 2017;43:49-58.
8. Sartori S, Tombesi P. Emerging roles for transthoracic ultrasonography in pleuropulmonary pathology. World J Radiol. 2010; 2:83-90.
9. Lumb P, Karakitsos D eds. Section VI General Chest Ultrasound. Critical Care Ultrasound. (Philadelphia: Elsevier, 2015) pp 105-137.
10. Irwin Z, Cook JO. Advances in Point-of-Care Thoracic Ultrasound. Emerg Med Clin North Am. 2016;34:151-157.
11. Sekiguchi H. Tools of the Trade: Point-of-Care Ultrasonography as a Stethoscope.

Semin Respir Crit Care Med. 2016;37:68-87.

2. Miller DL. Induction of pulmonary hemorrhage in rats during diagnostic ultrasound. Ultrasound Med Biol. 2012;38:1476-1482.
3. Miller DL, Dou C, Raghavendran K. The dependence of thresholds for pulmonary capillary hemorrhage on diagnostic ultrasound frequency. Ultrasound Med Biol 2015;41:1640-50.
4. Miller DL. Mechanisms for Induction of Pulmonary Capillary Hemorrhage by Diagnostic Ultrasound: Review and Consideration of Acoustical Radiation Surface Pressure. Ultrasound Med Biol. 2016;42:2743-2757.
5. Miller DL, Dou C, Raghavendran K. Anesthetic techniques influence the induction of pulmonary capillary hemorrhage during diagnostic ultrasound in rats. J Ultras Med. 2015;34:289-297.
6. Miller DL, Dou C, Dong Z, Raghavendran K. The Influence of Dexmedetomidine on Ultrasound-induced Pulmonary Capillary Hemorrhage in Rats. Ultrasound Med Biol. 2016;42:964-970.
7. Flecknel P. Laboratory Animal Anaesthesia. London: Academic Press, 2009.
8. Bihari S, Wiersema UF, Schembri D, De Pasquale CG, Dixon DL, Prakash S, Lawrence MD, Bowden JJ, Bersten AD. Bolus intravenous 0.9% saline, but not 4% albumin or 5% glucose, causes interstitial pulmonary edema in healthy subjects. J Appl Physiol (1985). 2015;119:783-792.
9. Teke Z, Adali F, Kelten EC, Enli Y, Sackan KG, Karaman K, Akbulut M, Goksin I. Mannitol attenuates acute lung injury induced by infrarenal aortic occlusion-reperfusion in rats. Surg Today. 2011;41:955-965.

0. Manenti A, Botticelli A, Buttazzi A, Gibertini G. Acute pulmonary edema after over-infusion of crystalloids versus plasma: histological observations in the rat. Pathologica. 1992;84:331-334.

1. Amouzadeh HR, Sangiah S, Qualls CW Jr, Cowell RL, Mauromoustakos A. . Xylazine-induced pulmonary edema in rats. Toxicol Appl Pharmacol. 1991;108:417-427.

2. Amouzadeh HR, Qualls CW Jr, Wyckoff JH 3rd, Dzata GK, Sangiah S, Mauromoustakos A, Stein LE. Biochemical and morphological alterations in xylazine-induced pulmonary edema. Toxicol Pathol. 1993;21:562-571.

Table 1. Measured in situ exposure parameters for the on screen Mechanical Index (MI_{os}) settings used here, including peak rarefactional pressure amplitude (PRPA), peak mean pressure amplitude (PMPA), and spatial peak pulse average intensity (I_{SPPA}).

Setting MI_{os}	PRPA MPa	PMPA MPa	I_{SPPA} W cm^{-2}
0.9	2.13 ± 0.21	2.77 ± 0.18	170 ± 20
0.7	1.71 ± 0.19	2.22 ± .016	114 ± 15
0.52	1.34 ± 0.13	1.68 ± 0.14	75 ± 13
0.37	1.02 ± 0.11	1.22 ± 0.11	40.5 ± 7.4
0.21 (aiming)	0.55 ± 0.06	0.62 ± 0.06	10.4 ± 2.0

Table 2. Measured physiological parameters for the four different conditions with and without xylazine. The mean blood pressure (MBP) is given in mm Hg before (bef) and after (aft) infusion, heart rate in beats per minute (bpm), and blood volume increase as a percentage (BV + %).

	Telazol only				Telazol + xylazine			
Parameter	None	Saline	Mannitol	Albumin	None	Saline	Mannitol	Albumin
n	18	5	5	5	24	10	20	20
Weight g	255 ± 9	261 ± 19	257 ± 25	262 ± 26	251 ± 16	268 ± 18	253 ± 16	258 ± 24
HR bpm	437 ± 58	415 ± 36	410 ± 48	420 ± 28	249 ± 27	295± 39	316 ± 49	322 ± 39
SpO_2 %	93 ± 5	88 ± 6	84 ± 9	89 ± 4	77 ± 9	79 ± 12	82 ± 8	76 ± 8
MBP bef	-	126 ± 28	115 ± 19	113 ± 18	-	82 ± 20	78 ± 14	94 ± 28
MBP aft	-	109 ± 36	96 ± 18	99 ± 17	-	81 ± 11	81 ± 14	78 ± 15
Wet/Dry	4.3 ± 0.2	4.1 ± 0.1	4.5 ± 0.6	4.9 ± 0.3	4.2 ± 0.2	4.5 ± 0.3	4.5 ± 0.3	4.8 ± 0.4
BV + %	-	23 ± 9	31 ± 7	37 ± 14	-	23 ± 11	30 ± 13	34 ± 13

Table 3. Pulmonary capillary hemorrhage results for diagnostic ultrasound scanning at the indicated MI_{os}: US CTA, ultrasound image comet tail artifact width; CTA %, the percentage of CTAs in the bright-line lung surface image. The intercept for the linear regression on the means (see Fig. 2) approximates the threshold PRPA for the condition with the corresponding coefficient of determination (r^2). Statistical significance relative to the sham (n=6) indicated by *. The linear regression intercepts are the apparent thresholds for PCH; regressions were not determined (ND) for the single data.

Condition	MI_{os}	US CTA mm	CTA %	Positive Proportion	Area mm^2	Intercept MPa	r^2
TO none	0.9	2.7±2.9	26±28	5/6	1.3±1.0*		
TO none	0.7	0.8±1.3	8.7±14.7	2/6	0.2±0.3	1.6	0.37
TO none	0.52	0	0	0/6	0		
TX none	0.9	8.4±2.3	87±24	6/6	4.6±1.3*		
TX none	0.7	2.3±2.8	36±30	4/6	1.2±1.9		
TX none	0.52	0.4±0.7	4.3±6.8	2/6	0.08±0.14	1.4	0.67
TX none	0.21	0	0	0/6	0		
TO Sal	0.9	0.2±0.5	2.0±4.4	1/5	0.37±0.83	ND	
TX Sal	0.9	1.7±1.9	19±21	3/5	0.5±0.6		
TX Sal	0.7	0.2±0.5	2.4±5.3	1/5	0.01±0.03	1.7	0.33
TO Man	0.9	0.4±0.8	3.0±6.7	1/5	0.8±1.8	ND	
TX Man	0.9	4.4±3.4	44±36	4/5	4.2±3.6*		
TX Man	0.7	4.5±2.3	46±23	5/5	2.6±2.2*		
TX Man	0.52	0.9±1.2	9.9±14.5	1/5	0.3±0.7	1.3	0.33
TX Man	0.37	0	0	0/5	0		
TO Alb	0.9	0.4±1.0	3.8±4.5	1/5	2.1±4.6	ND	
TX Alb	0.9	5.8±3.1	57±28	5/5	7.3±4.7*		
TX Alb	0.7	2.5±3.4	25±31	3/5	3.1±4.0		
TX Alb	0.52	1.0±1.7	9.5±15.4	2/5	1.3±2.1	1.2	0.45
TX Alb	0.37	0	0	0/5	0		

Figure Captions

Figure 1. The pulmonary capillary hemorrhage areas measured on the lung surface for the MI_{os}=0.9 groups. The * signifies means which are statistically significantly different from the sham. The fraction above each bar is the positive fraction for the group. The results for saline infusion were remarkably low for both the Telazol only (TO) and Telazol plus xylazine anesthesia (TX), which was significantly less than the TX group with no infusion ($p<0.001$). For TX, the no-infusion (none), mannitol infusion and albumin infusion results were all about the same. However, the mannitol and albumin infusions significantly enhanced the PCH relative to saline infusion.

Figure 2. Results for the pulmonary capillary hemorrhage areas for the exposure response tests: TO, Telazol only anesthesia; TX, Telazol plus xylazine anesthesia. The zero crossing values of the linear regressions provide values of the PCH thresholds, see Table 3. The saline infusion eliminated the enhancement effect of the xylazine.

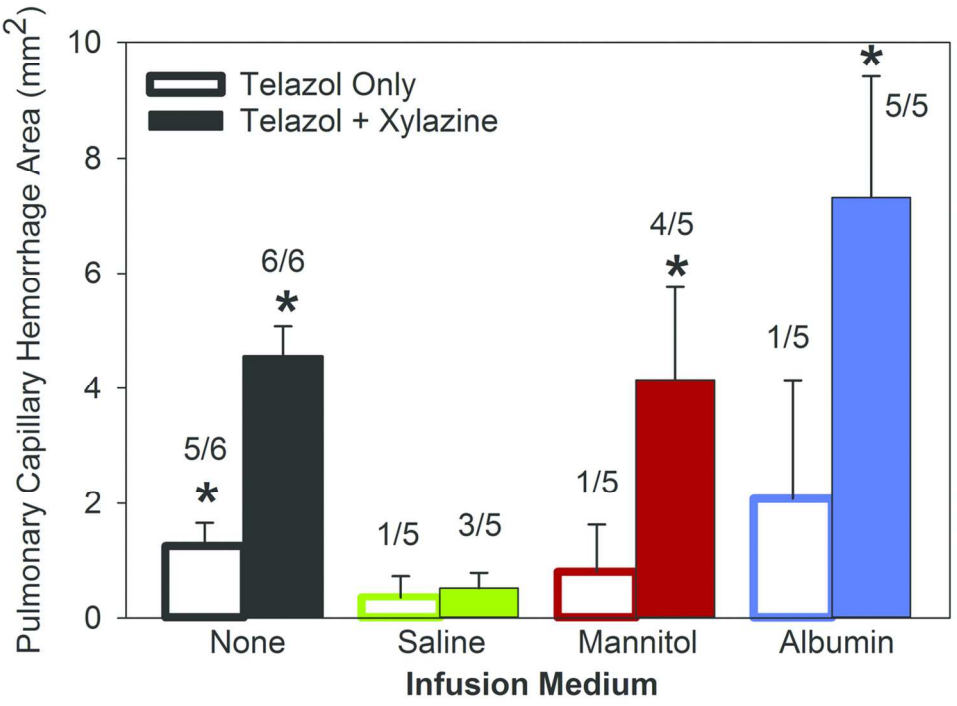

Figure 1. The pulmonary capillary hemorrhage areas measured on the lung surface for the MIos=0.9 groups. The * signifies means which are statistically significantly different from the sham. The fraction above each bar is the positive fraction for the group. The results for saline infusion were remarkably low for both the Telazol only (TO) and Telazol plus xylazine anesthesia (TX), which was significantly less than the TX group with no infusion ($p<0.001$). For TX, the no-infusion (none), mannitol infusion and albumin infusion results were all about the same. However, the mannitol and albumin infusions significantly enhanced the PCH relative to saline infusion.

59x44mm (600 x 600 DPI)

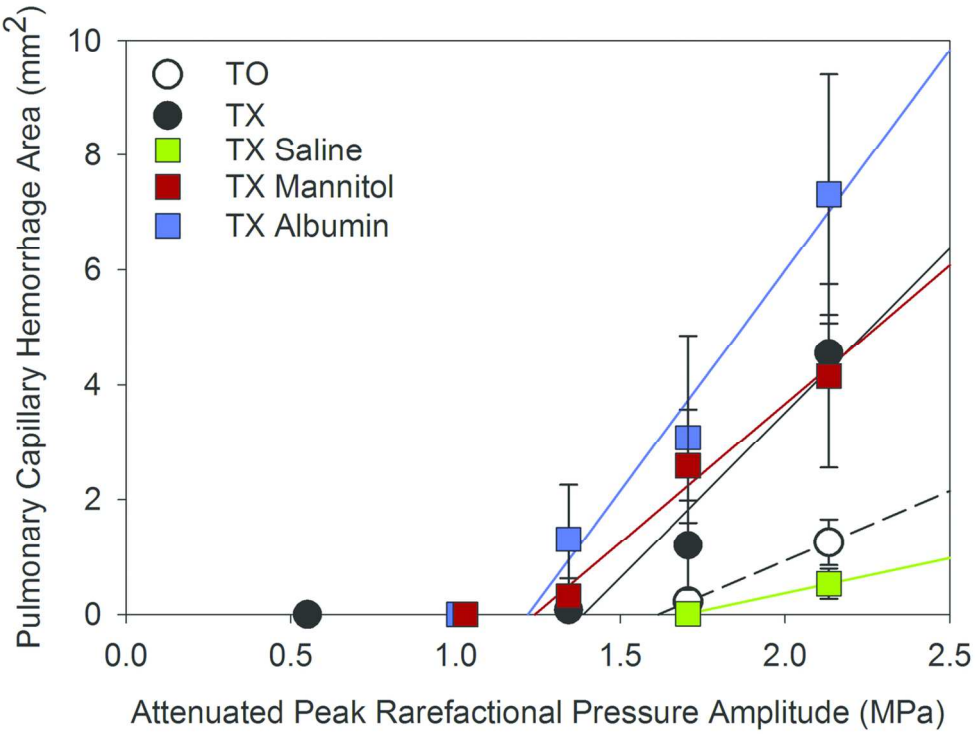

Figure 2. Results for the pulmonary capillary hemorrhage areas for the exposure response tests: TO, Telazol only anesthesia; TX, Telazol plus xylazine anesthesia. The zero crossing values of the linear regressions provide values of the PCH thresholds, see Table 3. The saline infusion eliminated the enhancement effect of the xylazine.

61x48mm (600 x 600 DPI)