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Hepatocyte Injury Induced by Contrast Enhanced Diagnostic Ultrasound

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ABSTRACT

Objectives: Contrast enhanced diagnostic ultrasound (CEDUS) has a potential to induce localized biological effects. The potential for CEDUS bioeffects in liver were researched, with guidance from a report by Yang et al. [1].

Methods: Contact and standoff scanning was performed for 10 min with a diagnostic ultrasound phased array at 1.6 MHz during bolus injection or infusion of contrast agent at a high dose. The impact of the imaging on rat liver was investigated by measuring enzyme release, microvascular leakage and staining of injured hepatocytes.

Results: The results demonstrated liver enzyme release at 30 min indicating liver injury and elevated extraction of Evans blue dye, indicating microvascular leakage. In addition, Evans blue and trypan blue vital-staining methods revealed scattered stained cells within the ultrasound scan plane. For the Evans blue method, fluorescent cell counts in frozen sections were greatest for standoff exposure with contrast infusion. The count decreased strongly with depth for bolus injection, which was probably reflective of the high attenuation noted for this agent delivery method.

Conclusions: The results qualitatively confirm the report by Yang et al., and additionally demonstrate hepatocyte vital staining. Research is needed to determine the threshold for the effects and the contrast agent dose-response.

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Key Words: diagnostic ultrasound adverse effects, ultrasonic cavitation biology, liver ultrasound imaging, contrast enhanced diagnostic ultrasound

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INTRODUCTION

Ultrasound contrast agents are used to enhance images with poor vascular resolution or detail [2]. Introduced in the 1990s, the agents have been approved for use in visualizing the left ventricle and aiding endocardial border delineation for patients with sub-optimal echocardiograms. In addition, many other applications have been explored. Recently, contrast enhanced diagnostic ultrasound (CEDUS) of liver has been established as a useful method [3]. The evaluation of incidental findings of focal liver lesions appears to be an especially valuable application [4-6].

Ultrasound contrast agents are suspensions of stabilized microbubbles. For example, Definity® (perflutren lipid microsphere injectable suspension, Lantheus Medical Imaging, Inc., N. Billerica, MA USA) is a solution of lipids supplied in a vial with perflutren (perfluoropropane) gas, which is shaken (using a dedicated vial shaker) to produce a suspension of microbubbles with a lipid skin. Definity is no longer sold for research purposes, but a replacement contrast agent (RCA) can be produced for research using a formula mimicking the Definity formula, as described previously [7]. The contrast-agent microbubbles are coincidentally small enough to circulate with the blood and have sizes suitable for strong interaction with diagnostic ultrasound pulses [8]. Diagnostic ultrasound pulses can activate the microbubbles sufficiently to nucleate ultrasonic cavitation, a well known mechanism for biological effects. The benchmark effect is injury to capillaries as the microbubbles pulsate violently. This leads to a

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progression of local damage with increasing pulse pressure amplitude, including endothelial cell injury, capillary leakage, petechial hemorrhage and injury to adjacent parenchymal cells. The resulting microlesion can then elicit fibrin formation and inflammatory responses. This potential for patient injury from CEDUS presents a safety issue which must be addressed to assure the performance of CEDUS without risk of cavitation-induced patient injury.

Microvascular injury and microlesions have been reported in several different tissues and organs including: intestine [9], cremaster muscle [10], abdominal muscle [11], heart [12, 13], kidney [14, 15], and pancreas [16]. The effects occur above specific pulse pressure amplitudes, i. e. thresholds, and the threshold for petechial hemorrhage increases approximately in proportion to ultrasound frequency in kidney [17] and heart [18], with minimal or no effects for CEDUS above about 5 MHz within the limits of diagnostic ultrasound.

Liver has received relatively little research attention regarding the cavitation safety issue. Shigeta et al. [19, 20] studied possible bioeffects in rat liver scanned with an Acuson Sequoia 512 diagnostic ultrasound machine with linear and curved arrays using Mechanical Index (MI) values of 0.7 or 1.8 (an exposure index related to cavitation potential) with continuous scanning for 1 min. A late phase exposure (10 s) was also performed to scan microbubbles taken up by Kupffer cells 5 min after administration. The contrast agents Levovist (air filled with palmitic acid stabilization,

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Schering AG) and DD-723 (later named Sonazoid, perfluorobutane with phosphatidyl serine stabilization, Daiichi Sankyo Co.) were investigated using histology in light and electron microscopy. Endothelial injury and platelet aggregates were found in exposed rat livers. Vacuolization was seen in hepatocytes, and microbubbles were observed inside Kupffer cells, likely owing to phagocytosis. In addition, increased platelet activation was observed for *in vitro* testing in ultrasound plus contrast agent groups. The exposures had two aspects, which would tend to minimize cavitational bioeffects; a relatively high ultrasound frequency (either 8 or 12 MHz) and continuous exposure (which can destroy microbubbles before they reach the focal region). The platelet activation seen *in vitro*, especially with Levovist may not actually translate to the *in vivo* situation.

Yang et al [1] utilized a GE Vivid 7 Dimension ultrasound machine with S4 transducer at 1.5 MHz to evaluate the safety of ultrasound-targeted microbubble destruction (related to use of diagnostic ultrasound as means for gene delivery to liver cells). The probe was hand-held on the depilated abdomen of rats with a 3 cm image depth. Exposure to the liver was performed by alternating a low viewing MI of 0.08, then switching to a “flash” mode at a high MI of 1.0, which gave alternating destruction and refill of microbubbles, and continued for 10 min. The contrast agent Zhifuxian was used as a 500 µL/kg bolus dose. This agent had lipid stabilization and perfluoropropane gas, which was prepared by shaking to yield 4-9 (10^9) ml^{-1} microbubbles of 2.1 µm

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mean diameter [21]. Groups included a control, agent only, ultrasound only and ultrasound plus agent with assessment at 0 hr, 0.5 hr, 12 hr and 24 hr time points. Evans blue dye was used as a tracer for vascular permeability in optical microscopy and tissue extraction, and lanthanum Nitrate was used as a tracer for electron microscopy. In addition the liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured in serum samples as a measure of liver injury. The ultrasound plus microbubble groups had Evans blue extravasation into the parenchyma, and lanthanum detectable in the cytoplasm. The AST and ALT was elevated at 0.5 hr, but returned to normal at 24 hr. Overall, the results suggested CEDUS increased capillary and cell membrane permeability with some hepatic toxicity.

The purpose of this present study was to evaluate CEDUS induced bioeffects in rat liver. The Yang et al [1] study was used as the initial model because it appears to be the only account of CEDUS induced liver injury. Our GE Vivid 7 Dimension machine was used with our replacement contrast agent (similar to Definity). Results were generally supportive of the Yang et al. [1] study and also indicated hepatocyte injury by Evans blue staining after application of intermittent exposure protocols similar to our previous heart and kidney research.

METHODS

Animal preparation

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This *in vivo* research was conducted with the approval of the Institutional Animal Care and Use Committee at the University of Michigan. Male rats (Sprague Dawley, Charles River Laboratories, Wilmington MA) with a mean weight of 317 ± 36 gm were used in this study, as detailed below. Six were lost from the study due to anesthetic death or technical problems. The rats were anesthetized via intraperitoneal injection of 40 mg/kg pentobarbital. The abdomen was shaved and depilated to maximize ultrasound transmission. A 24 gauge cannula was inserted into a tail vein for intravenous injections and infusion of contrast agent. Rats were placed in the dorsal decubitus position on a warming pad with ultrasound transmission gel on the abdomen over the liver. Rats were scanned either with the ultrasound probe directly on the abdomen or with the probe above the abdomen using a small water-filled standoff to place the focal zone within the liver. The standoff design utilized a thin polyethylene window for transmission. When Evans blue dye injection was not used, rats were checked for heart rate and SpO_2 (percentage of oxygen saturation of hemoglobin in peripheral blood), which averaged 325 ± 18 beats per min and 81 ± 3 %, respectively.

Diagnostic Ultrasound

Yang et al. [1] used a GE Vivid 7 Dimension machine, with an S4 transducer placed on the abdomen. The second harmonic B mode was used with 1.5 MHz transmit and 3.2 MHz receive frequencies. A 3 cm image and focus depth were used with an intermittent exposure scheme. Low MI images were used to visualize the arrival of

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contrast agent microbubbles, and then the mode was switched to “flash” mode at the maximum MI=1.0. The high and low modes were switched rapidly to observe alternating phases of microbubble destruction and re-fill in the imaging plane. The total scan time was 10 min.

For this study, a 3S phased array probe (GE Vivid 7 Dimension, GE Vingmed Ultrasound, Horten, Norway) was used with two different procedures to exposure the right medial lobe of the liver in a transverse plane. For contact exposure (CE), a 3 cm image and focal depth was used. Our machine had an Octave mode with 1.6 MHz transmit and 3.2 MHz receive frequencies, but did not have a “flash” mode. The machine was therefore switched quickly (approximately 20 s cycle time for refill) between low MI=0.1 and a maximum MI=1.0 at 25.2 fps, continued for 10 min. This contact method was somewhat inconsistent due to the manual operation. For stand-off exposure (SE), the image depth was 8 cm, the focus depth was 5 cm, and the anterior liver lobe was located at the measured optimal focus of 3.8 cm by adjusting the position of the probe. This arrangement was previously used for studies of contrast-related bioeffects in rat heart (Miller et al. 2016). The scan pulse repetition frequency was 4.1 kHz, and the pulse duration was 1.52 μs. A time trigger-signal at 1 Hz was directed into the ECG input and used to set a 10 s intermittent scan interval. The intermittent triggering allowed refill of the tissue with contrast agent between triggered dual images. This dual image method can display contrast microbubble destruction as a loss of

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contrast enhancement for the second image.

The pulse-pressure waveforms were measured in a water bath using a calibrated hydrophone with 0.2 mm sensitive spot (HGL0200, Onda, Sunnyvale, CA, USA), and the peak rarefactional pressure amplitude (PRPA) was determined at two depths 0.5 cm apart, which approximately corresponded to the anterior and posterior surfaces of the scanned liver lobe. The depths were 0.5 cm and 1.0 cm for the CE condition, and 3.8 cm and 4.3 cm for the SE condition. Values are listed in Table 1 for the pulse parameters, and -6 dB scan widths and thicknesses. For comparison, the dimensions of the active region of the probe were 1.5 cm (perpendicular to the scan plane) and 2.1 cm (in the scan plane). Pulse durations were calculated from the time-intensity integral of the pulse waveform. The intermittent scans were performed at the maximum power setting of 0 dB (MI=1.3). The standoff with intermittent imaging was superior to the contact method both in terms of the consistency of exposure and in terms of the maximum pulse parameters delivered. The contact method was only used for a few tests with Evans blue.

Ultrasound Contrast Agent

The ultrasound contrast agent was a laboratory replacement for Definity® (perflutren lipid microsphere injectable suspension, Lantheus Medical Imaging, Inc., N. Billerica, MA USA), which is no longer sold for research purposes. A replacement contrast agent (RCA) for Definity was created using a formula mimicking the Definity

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formula, as described previously [7]. The lipid mixture was sterilized and aliquoted into empty sterile Definity vials with the headspace filled with octafluoropropane (HC-218, PurityPlus, Metro Welding Supply, Detroit MI USA). The vials were shaken for 45 s in a VialMix (DuPont Pharmaceuticals Co., Billerica MA USA) before use to produce the suspensions of stabilized microbubbles. The RCA microbubbles were assessed using a Coulter counter (Multisizer 4, Beckman Coulter, Inc. Indianapolis IN USA) [18]. The mean diameter was slightly larger at 1.8 ± 0.11 μm diameter than actual Definity microbubbles at 1.6 ± 0.04 μm diameter, but the concentration of microbubbles in our tests was not significantly different from Definity at $3.7 \pm 0.3\ 10^{9}\ ml^{-1}$. The RCA was prepared each day, diluted with sterile saline and administered as a bolus dose of 500 μl/kg, to reproduce the dosage used by Yang et al. [1], or as an infusion of 50 μl/kg/min for the 10 min scans. Bolus injection was used for CE exposures. For SE exposures, both bolus and infusion dosing was used in different groups. These dosages were high compared to clinically recommended doses of Definity (10 μl/kg bolus, or about 3.7 μl/kg/min infusion for a 70 kg human). For sham exposure, the rat was scanned for 5 min using the maximum ultrasound (0 dB) setting, and then the contrast agent was infused for 10 min with the ultrasound off. Examples of the dual images are shown in Fig. 1 before and after contrast delivery. The bolus injection produced large contrast enhancement at the surface but shadowing, indicative of increased attenuation, with depth.

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Experimental Plan and Measured Endpoints

In preliminary testing, the ultrasound scanning geometry was established so that the scan plane was located at about the same position for each rat. However, there was no identifiable indication of a contrast enhanced ultrasound-induced effect on the scanned liver samples, in contrast to the clear indications of petechiae which can be demonstrated in heart [22] or kidney [23]. Groups of rats had blood samples taken before and 30 min after scanning for evaluation of liver enzymes, in an effort to detect liver injury, as reported by Yang et al. [1]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were both measured (Activity Assay Kits, Sigma-Aldrich.com, St. Louis MO USA). The liver was perfused with saline and then with neutral buffered formalin for fixation. Histology slides were made of tissue slabs cut to cross-section the scan plane to search for injury.

Yang et al. [1] utilized Evans blue dye to test for microvascular leakage. In our studies of bioeffects of myocardial contrast echocardiography, Evans blue has been useful to show microvascular leakage [13], and also to stain lethally injured cardiomyocytes [23]. The cardiomyocyte staining can be evaluated by fluorescence microscopy to score numbers of cavitational microlesions, which consist of a petechial hemorrhage and one or more injured cardiomyocytes [18]. Evans blue dye was prepared at 50 mg/ml in saline and injected before ultrasound scanning at 50 mg/kg. Liver samples were obtained 30 min post exposure after perfusion clearance of the

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circulation with 300 ml heparin saline. For evaluation of microvascular leakage, liver samples were weighed, minced and extracted in formamide. After centrifugation, the supernatant was measured by spectrophotometry at 620 nm to determine Evans blue content. In addition, blue stained cells were evident on the liver surface within the scan plane. This effect was scored in frozen sections by fluorescence microscopy. Three sections cut to cross-section the scan plane (spaced 1.5 mm apart) from each rat liver were scored by counting fluorescent cells in 5 adjacent 10x fields of view from the anterior surface inward to the posterior surface of the sample. The scoring was performed on photomicrographic images (Spot Flex, Diagnostic Instruments Inc., Sterling Heights, MI USA) for fixed exposure times set to maximize the conspicuity of the stained cells (relative to the background).

The Evans blue appeared to stain the cytoplasm of hepatocytes, which was not clearly indicative of lethal injury. A second method of vital staining was tried using trypan blue vital stain, based on staining methods for liver injury [24]. After perfusion with heparin saline, perfusion was continued with 120 ml trypan blue solution (10:1 dilution by heparin saline of Trypan Blue Stain 0.4%, Gibco, Fisher Scientific, USA) followed by 120 ml heparin saline. The liver samples showed stained cells in the scan plane after this procedure. Histological observation was also tried using perfusion and immersion fixation with neutral buffered formalin and preparation of histological paraffin sections without only Eosin stain, as reported by [24]. However, the histology slides did not retain

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the Trypan blue staining sufficiently for confident scoring, and only qualitative observation of fresh samples was performed.

The numbers of rats used for the different exposures and tests are listed in Table 2. Results were evaluated by comparisons of two means of measurements in different groups of rats. Statistical analysis was performed using SigmaPlot for Windows V. 11.0 (Systat Software Inc., San Jose CA, USA). The Mann-Whitney Rank Sum test and the Student t-test were used for the comparisons of un related samples, while pared tests were used for comparing before-and-after tests. Statistical significance was assumed at $p<0.05$.

RESULTS

Enzyme assays were performed for the standoff method using bolus and infusion contrast delivery. Results for ALT are shown in Fig. 2 as before and after comparisons measured in sham and exposed samples. The ALT for exposure with contrast infusion was significantly elevated relative to shams ($p<0.05$). For AST, the enzyme activity was significantly increased for both contrast infusion and bolus, and the after exposure results was significantly elevated above the before results for contrast bolus (Fig. 3). The enzyme results demonstrated enzyme release indicative of liver cell injury.

The extraction of Evans blue dye from exposed liver samples was performed for all the methods. Results are shown in Fig. 4. Only the exposure with contrast infusion

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was significantly increased ($p<0.05$) above both its respective control samples (out of the beam path) and sham exposed samples. These results indicated that the contact exposure method was relatively ineffective. In addition, the standoff exposure with contrast infusion appeared to be more effective than exposure with the bolus injection of contrast agent.

In preliminary tests, freshly excised livers were examined and fixed for histology with hematoxylin and eosin staining to find any clear indications of injury, such as petechiae, which are seen for contrast enhanced diagnostic ultrasound of other organs. No bioeffects could be clearly identified, even after perfusion clearance of the blood in the circulation. However, both the Evans blue and the trypan blue methods revealed a band of scattered stained cells corresponding to the scan plane in fresh samples. Outside this region, stained cells were essentially absent confirming the effect of ultrasound exposure in the scan plane. Examples of the appearance of the freshly excised livers are shown in Fig. 5. The Evans blue stained cells showed low contrast for in brightfield, but the trypan blue gave a readily discernable bluish band, which results from numerous blue stained cells, in the scan plane.

In frozen sections, the Evans blue stained cells could be readily identified by the red fluorescence of the Evan blue, as shown in Figure 6. There was a substantial background in some samples, in which cells were outlined in red fluorescence possibly owing to incomplete perfusion removal of the dye. However, the positively stained cells

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were distinct with bright fluorescence, which filled in the entire cell. In fresh tissue slices, the individual trypan blue stained cells were readily identified by strong blue staining of the cell nuclei, as shown in Figure 6 at the same scale as the Evans blue frozen section image. Qualitatively, the scattered distribution and number of effected cells appears to be about the same for the two staining procedures. Unfortunately, the fresh hand-cut slices were thick and uneven so that the two stain methods could not be quantitatively compared. As noted in methods, the histological processing of the trypan blue stained samples and staining with eosin only gave greatly diminished conspicuity of the stained cells (compared to fresh samples), and these could not be scored with confidence. The Evans blue stained cells could be quantitatively counted and the fluorescent cell counts are presented in Fig. 7. The CE method gave relatively low results compared to the SE bolus method ($p<0.01$ for the anterior position). In addition, the SE method with contrast infusion appeared to be more efficacious relative to the contrast bolus, particularly for deeper portions of the liver sample ($p<0.05$).

DISCUSSION

Contrast enhanced diagnostic ultrasound (CEDUS) has a potential for induction of localized biological effects, which have been reported, for example, in muscle, heart, and kidney. The effects include microvascular leakage, petechial hemorrhage and lethal injury of parenchymal cells at the highest pulse pressure amplitudes. Liver is a good

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candidate for CEDUS examination, but little research has been reported for potential bioeffects in liver. In this study, the potential for bioeffects was researched for rat liver, following a report by Yang et al. [1] of CEDUS induced liver enzyme release, and vascular permeabilization.

The methods of Yang et al. [1] were used as much as possible, although our ultrasound machine was not capable of duplicating their exposures. The contact exposure method placed the liver lobe in the nearfield of the probe, and delivered reduced exposure levels compared to the focal zone (Table 1). Our study included mostly focal zone exposure using a standoff, which provided higher exposure levels in the anterior liver lobe, and was similar to previous research in other organs. The large bolus dose produced high attenuation, evident in the ultrasound images (Fig. 1). In our study we added exposure with 10 s intermittent scans and infusion of the contrast agent which reduced attenuation and shadowing.

The results demonstrated enzyme release for 30 min samples (Figs. 2 and 3). In addition, extraction of Evans blue dye from the exposed tissue showed a significant increase for the SE method within contrast infusion (Fig. 4). Therefore, the findings of Yang et al. [1] were confirmed qualitatively, even though the methods could not be quantitatively compared.

In addition, we found that Evans blue and trypan blue vital-staining methods revealed a band of scattered stained cells corresponding to the ultrasound scan plane

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(Fig. 5). The two methods appeared to detect about the same distribution and number of stained cells (Fig. 6), which indicates lethal injury of some hepatocytes. The level of injury was small, less than 1 % of cells, but nevertheless quite clearly defined. For the Evans blue method a key step was perfusion clearance of the blood. Even lengthy perfusion clearance left a substantial background in the form of a fluorescent border around many hepatocytes. This was not a bioeffect of the CEDUS, and may simply show remaining Evans blue, possibly within the space of Disse, not cleared by the perfusion blood clearance step. Although the trypan blue method was not amenable to quantitative histological characterization, the Evans blue method gave quantitative fluorescent cell counts as a function of depth into the liver samples (Fig. 7). These results showed a strong decrease with depth for bolus injection, probably reflective of the high attenuation noted for this agent delivery method.

Given the high contrast doses, the effects in this study seem relatively small, for example, compared to heart [18]. However, the dose was sufficient to produce substantial attenuation, possibly reducing the exposure impact. In addition, the low microvascular impact may be due to the nature of the sinusoidal capillaries present in liver, which are large (7-15 μm in diameter) and lined by discontinuous endothelium [25]. Recent research indicates the capillaries, for example, in kidney are injured by the action of the largest microbubbles in contrast agents, and that microbubble expansion sufficient to stress capillary walls is required for capillary rupture [26, 27]. The minimal impact on

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liver sinusoids may be related to the limited number of large microbubble sizes available after passage through the lungs. Further work has been pursued to better define the bioeffect response both in terms of contrast agent dosage and microbubble sizes, and the ultrasound pulse amplitude threshold for the hepatocyte injury, and results will be reported in a separate paper.

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Table 1. The results for measurement of ultrasound pulse parameters in a water bath. The contact method used 3 cm focus and depth settings with 25.2 frames per s, while the standoff methods used a 5 cm focus and 8 cm depth with 56.3 frames per s. The scan plane area was determined as -6 dB positions crossing the scan plane (thickness) and along the scan plane (length).

Method	Test Depth cm	Pulse Dur. μs	PRPA MPa	PRPA/$f^{1/2}$ MPa/$MHz^{1/2}$	Thickness mm	Length cm
Contact	0.5	1.6	0.86	0.70	12.7	1.55
Contact	1.0	1.9	1.12	0.90	10.0	1.95
Standoff	3.8	1.5	2.47	2.0	5.0	6.9
Standoff	4.3	1.5	2.40	1.9	5.0	7.7

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Table 2. Numbers of rats used for the various exposures and evaluation tests; np, not performed.

	Bolus		Bolus		Infusion	
Test	CE	CE sham	SE	SE sham	SE	SE sham
Enzymes	np	np	7	5	6	5
EB extract	6	6	8	np	8	6
EB count	4	4	6	np	11	10
Trypan blue	np	np	6	np	6	np

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Figure Captions:

Figure 1. Dual ultrasound images during exposure using the standoff method with bolus injection (top row) and infusion (bottom row) of contrast agent. The left column shows the pre-injection image with the liver anterior liver lobe about 5 mm thick just below the abdominal wall. Shadows are produced by the spine and by intestinal gas. When the contrast agent is administered (middle column), the image brightness is enhanced at the anterior liver, with attenuation shadowing of deeper tissues, especially for the bolus injection. At the end of the 10 min exposure (right column), the contrast effect is greatly reduced for the bolus injection, but still present for the infusion. The contrast brightness is somewhat reduced for the right side image of each pair due to microbubble destruction.

Figure 2. Results for measurements of plasma alanine aminotransferase (ALT) in blood samples taken before and after sham or exposure. The ALT was significantly increased ($p<0.05$) for the after exposure with infusion (#), relative to the sham.

Figure 3. Results for measurements of plasma aspartate aminotransferase (AST) in blood samples taken before and after sham or exposure. The AST was significantly elevated ($p<0.05$) for the after exposure sample with infusion relative to sham (#), and for the bolus injection for the exposed sample relative to both the sham (#) and the before exposure sample (*).

Figure 4. Results for the extraction of Evans blue from liver samples. For the standoff

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exposure with infusion of contrast agent (*), the extracted Evans blue was significantly elevated ($p<0.05$), relative to paired controls and to shams.

Figure 5. Stereo microscope images of freshly excised liver lobes after perfusion clearance of blood and dye solution: Evans blue (top) and trypan blue (bottom). The arrows indicate the position of the blue band of stained cells within the scan plane. Scale bars: 1 cm.

Figure 6. Images of the Evans blue stained cells for fluorescence microscopy in a frozen section (left) and trypan blue stained cells (right) for stereomicroscopy of a freshly cut sample. Both samples had standoff exposure with contrast infusion. Scale bars: 0.5 mm.

Figure 7. Results for Evans blue stained cells counted in fluorescent microscopy of frozen sections. Five fields of view were scored for each sample lobe, from the anterior surface (0 mm) inward toward the posterior side. The strong decrease of the score with depth for the bolus injection and standoff exposure probably reflects the attenuation produced by the high contrast agent dose (see Fig. 1).

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