Hemorrhage Near Fetal Rat Bone: Preliminary Results

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Abstract. High-intensity ultrasound has shown potential in treating many ailments requiring noninvasive tissue necrosis. However, little work has been done on using ultrasound to ablate pathologies on or near the developing fetus. For example, Congenital Cystic Adenomatoid Malformation (cyst on lungs), Sacrococcygeal Teratoma (benign tumor on tail bone), and Twin-Twin Transfusion Syndrome (one twin pumps blood to other twin) are selected problems that will potentially benefit from noninvasive ultrasound treatments. Before these applications can be explored, potential ultrasound-induced bioeffects should be understood. Specifically, ultrasound-induced hemorrhage near the fetal rat skull was investigated. An f/1 spherically focused transducer (5.1-cm focal length) was used to expose the skull of 18- to 19-day-gestation exteriorized rat fetuses. The ultrasound pulse had a center frequency of 0.92 MHz and pulse duration of 9.6 μs. The fetuses were exposed to 1 of 4 exposure conditions (denoted A, B, C, and D) in addition to a sham exposure. Three of the exposures consisted of a peak compressional pressure of 10 MPa, a peak rarefactional pressure of 6.7 MPa, and pulse repetition frequencies of 100 Hz (A), 250 Hz (B), and 500 Hz (C), corresponding to time-average intensities of 1.9 W/cm^2, 4.7 W/cm^2, and 9.4 W/cm^2, respectively. Exposure D consisted of a peak compressional pressure of 6.7 MPa, a peak rarefactional pressure of 5.0 MPa, and a PRF of 500 Hz corresponding to a time-average intensity of 4.6 W/cm^2. Hemorrhage occurrence increased slightly with increasing time-average intensity (i.e., 11% for A, 28% for B, 31% for C, and 19% for D with a 9% occurrence when the fetuses were not exposed). The low overall occurrence of hemorrhaging may be attributed to fetal motion (observed in over half of the fetuses from the backscattered echo during the exposure). The mean hemorrhage sizes were 3.1 mm^2 for A, 2.5 mm^2 for B, 2.7 mm^2 for C, and 5.1 mm^2 for D. The larger lesions at D may be related to these fetuses moving less as only 40% of the fetuses were observed moving for this exposure condition.

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INTRODUCTION

In addition to its long history in diagnostic application, ultrasound at high intensities has also been used in therapeutic applications. The main advantage of ultrasound in treating ailments has been its ability to noninvasively target the tissue region of interest. This is a great advantage when other treatment options, such as surgery, are not possible or greatly increase the risk to the patient. One application

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that has received relatively little attention is using ultrasound to ablate pathologies on or near the developing fetus. For example, Congenital Cystic Adenomatoid Malformation (cyst on lungs), Sacrococcygeal Teratoma (benign tumor on tail bone), and Twin-Twin Transfusion Syndrome (one twin pumps blood to other twin) are selected problems that will potentially benefit from noninvasive ultrasound treatments. Surgery for these ailments greatly increases the risk of pre-term delivery, thus increasing the potential for further complications after birth.

Before the use of ultrasound therapy for the fetus can be safely explored, potential ultrasound-induced bioeffects on the fetus should be better understood. Although considerable work has been done on ultrasound-induced thermal bioeffects[1-5], not all bioeffects may result from heating. Ultrasound-induced hemorrhage near the fetal skull, for example, is of particular interest due to an earlier study that claimed the hemorrhage cause was not due to heating[6]. The goal of this study was to provide more experimental evidence of hemorrhage near fetal bone resulting from ultrasound exposure to determine if the hemorrhage could at least be correlated with the time-average intensity of the ultrasound. If the damage does correlate with the time-average intensity, then the risk of hemorrhage can be estimated in the same way the risk of heating is assessed. Further studies may also demonstrate that the hemorrhage is a direct result of heating.

EXPERIMENT PROTOCOL

Pregnant rats with a gestational age of 18-19 days were anesthetized with ketamine hydrochloride (87.0 mg/kg) and xylazine (13.0 mg/kg) administered intramuscularly. The pregnant rats were placed in dorsal recumbency and their abdomens shaved. Incisions were made in the pelvic region and the tissue layers pulled back revealing the uterus containing the developing fetuses. A stand-off vessel containing degassed water (heated to 37ºC) was placed in contact with the uterus and mineral oil was used as a coupling agent on the uterine surface. The ultrasound beam axis for an f/1 (5.1-cm focal length) spherically focused transducer (center frequency of 0.92 MHz) was then visually aligned through the uterus over the developing fetal skull. The fetal skull was then positioned at the focus along the beam axis by maximizing the reflection at the focal depth from a low-amplitude pulse with a pulse duration (PD) of 12.3 μs (i.e., alignment exposure).

After alignment, each fetus was exposed to 1 of 4 ultrasound exposures (denoted A, B, C, and D) using PDs of 9.6 μs or a sham exposure for an exposure duration of 60 seconds. An overview of each of the exposure conditions including the alignment exposure are provided in Table 1.

TABLE 1. Overview of exposure conditions.

Exposure	p_c, p_r (MPa)	PRF	I_{TA}
Alignment, Sham	1.1, 1.1	25 Hz	6.9 mW/cm^2
A	10.2, 6.7	100 Hz	1.9 W/cm^2
B	10.2, 6.7	250 Hz	4.7 W/cm^2
C	10.2, 6.7	500 Hz	9.4 W/cm^2
D	6.7, 5.0	500 Hz	4.6 W/cm^2

For 3 of the 4 exposures (A, B, and C), the peak compressional pressure (p_c) and the peak rarefactional pressure (p_r) were the same; only the pulse repetition frequency (PRF) changed. If hemorrhage correlated with ultrasound pressure, all three of these exposures should result in the same occurrence and size of hemorrhage. Also, exposures B and D had approximately the same temporal-average intensity (I_{TA}). Hence, the hemorrhage occurrence and size for exposures B and D should be very similar if hemorrhage correlated with I_{TA}.

Thirty-six fetuses were used for each ultrasound level or sham exposure for a total of 180 fetuses. The exposure conditions for the fetuses were randomized. After the ultrasound exposure of the fetuses, the mother and fetuses were euthanized while under anesthesia and examined for tissue hemorrhage. In addition, two pregnant rats were anesthetized and euthanized without exteriorizing the fetuses (17 total) or exposing them to ultrasound; the fetuses were then assessed for hemorrhages. The individuals responsible for alignment of the transducer and assessing the hemorrhaging remained blinded to the exposure conditions until the completion of the study. The experimental protocol was approved by the University of Illinois Institutional Animal Care and Use Committee. The animals were housed in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) approved animal facility and provided food and water *ad libitum*.

RESULTS

The hemorrhage occurrence versus I_{TA} and p_r for the fetuses are shown in Figure 1. The solid line shows the total percentage of fetuses where hemorrhage was observed anywhere on the body of the fetus. The dashed line shows only the percentage of fetuses where hemorrhage was observed on the head of the fetus while excluding the fetuses that had hemorrhage elsewhere.

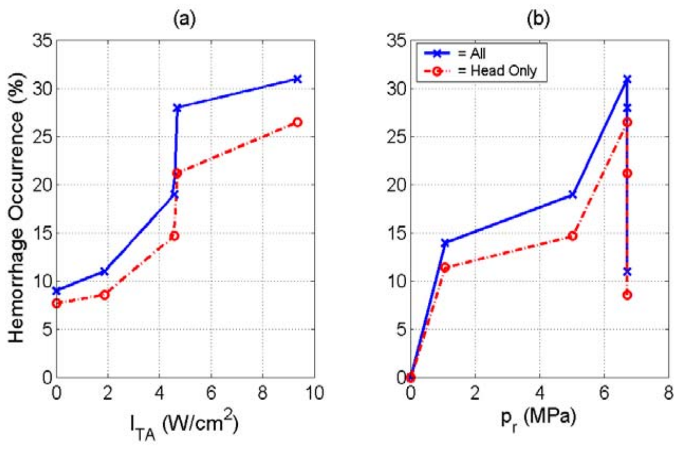

FIGURE 1. Hemorrhage occurrence versus (a) temporal-average intensity (I_{TA}) and (b) peak rarefactional pressure (p_r).

The results for an I_{TA} of zero (Figure 1a) include both the sham exposures (5/36 for solid line, 4/35 for dashed line) as well as the 17 fetuses (0/17 for both) that were assessed for hemorrhage without being exteriorized while alive. There is a monotonic increase in hemorrhage occurrence as I_{TA} increases indicating a dependence on I_{TA} while the hemorrhage occurrence does not correlate with p_r. Hemorrhage occurrence for the 4.6 W/cm^2 exposure (i.e., exposure D) is different from that for the 4.7 W/cm^2 exposure (i.e., exposure B), but the difference is on the same order as hemorrhage occurrence in the sham exposures.

In addition to hemorrhage occurrence, the damage can also be quantified by the hemorrhage area by approximating the region by an ellipse and measuring the major and minor axes. The largest (upper error bar), smallest (lower error bar), and mean hemorrhage area for the different exposure conditions are given in Figure 2. Although there are large variations in the size of the hemorrhage, the largest hemorrhages occur for exposure D. Hence, the size of the hemorrhage does not correlate with I_{TA} or the exposure pressure in this preliminary study.

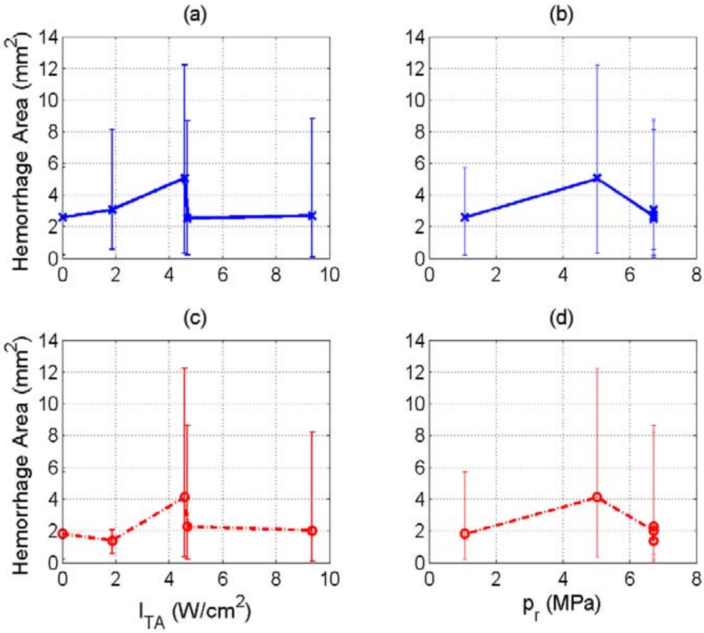

FIGURE 2. Hemorrhage area for (a) all locations versus I_{TA}, (b) all locations versus p_r, (c) hemorrhages that occur on the head versus I_{TA}, and (d) hemorrhages that occur on the head versus p_r.

DISCUSSION

Hemorrhage occurrence correlated with I_{TA} while hemorrhage area did not. The low overall hemorrhage occurrence was surprising but may be related to fetal motion. After positioning, the fetus could move, possibly even leave the path of the ultrasound beam completely. In the worse case scenario, an adjacent fetus could shift into the beam due to their proximity to one another in the uterus. This may explain hemorrhage occurrence in some of the "sham" exposures as well as the hemorrhage on locations other than the fetal head. Minimally, the same location on the fetus would not be exposed for the entire time if motion occurred.

Some motion could be detected by monitoring the backscattered waveform during the ultrasound exposure provided that the reflected waveform did not saturate the receiver. The receiver gain was set to monitor the alignment echoes and not the exposure echoes. In this study, motion was observed in 14/26 (54%) of the echoes that did not saturate the receiver for exposure A, 11/23 (48%) of the echoes that did not saturate the receiver for exposure B, 14/19 (74%) of the echoes that did not saturate the receiver for exposure C, and 11/28 (39%) of the echoes that did not saturate the receiver for exposure D. The mean hemorrhage area is plotted versus the percentage of perceived motion in Figure 3.

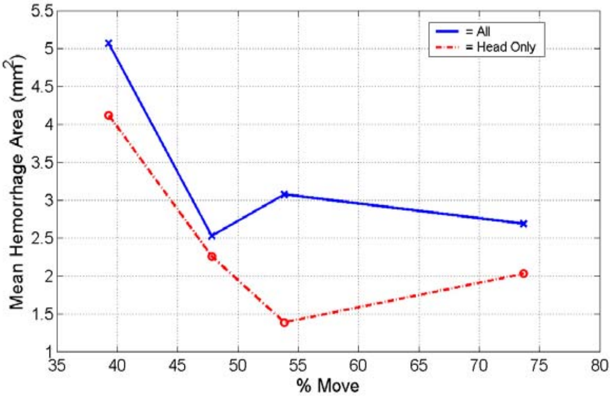

FIGURE 3. Hemorrhage area versus % of perceived motion.

Hence, the hemorrhage area appears to correlate with the amount of fetal motion. In the future, it may be better to partially remove the living fetus from the uterus so that fetal motion and position can be better controlled. It may then be possible to determine if hemorrhage area also has a dependence on I_{TA}.

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REFERENCES

1. Edwards, M.J., Shiota, K., Smith, M.S.R. et al., *Reproductive Toxicology* **9**, 411-425 (1995).
2. Horder, M.M., Barnett, S.B., Vella, G.J. et al., *Early Human Development* **52**, 221-233 (1998).
3. Barnett, S.B., *Ultrasound in Medicine & Biology* **26**, S68-S70 (2000).
4. Stone, P.R., Ross, I., Pringle, K. et al., *Fetal Diagn Ther* **7**, 26-30 (1992).
5. Duggan, P.M., Liggins, G.C., and Barnett, S.B., *Ultrasound in Medicine & Biology* **21**, 553-560 (1995).
6. Dalecki, D., Child, S.Z., Raeman, C.H. et al., *Ultrasound in Medicine & Biology* **25**, 1139-1144 (1999).