

Role of Pulse Repetition Frequency and Exposure Duration on the Superthreshold Behavior of Ultrasound-induced Lung Hemorrhage in Adult Mice and Rats

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Abstract - Superthreshold behavior for ultrasound-induced lung hemorrhage was investigated in 150 mice and 150 rats at 2.8 MHz to assess the role of pulse repetition frequency (PRF) and exposure duration (ED). Each species was divided into 15 exposure groups (10 per group) for a 3x5 randomized factorial design (3 EDs of 5, 10 and 20 s; and 5 PRFs of 25, 50, 100, 250 and 500 Hz). The *in situ* peak rarefactional pressure (12.3 MPa) and pulse duration (1.42 μ s) were the same for all ultrasonically exposed animals. Also, for both species, 15 sham-exposed animals were randomized into both studies, none of which had lesions. Factorial analysis of variance was used to evaluate effects of PRF and ED on the proportion of lesions, lesion depth and lesion surface area. The proportion of lesions in both species was related statistically to PRF and ED, with the exception that PRF in rats was not quite significant. The PRF, but not ED, significantly affected lesion depth in both species. Both PRF and ED significantly affected lesion surface area in mice, while neither affected area in rats. The PRF \times ED interaction (number of pulses) for these measures was not significant for either species. Species significantly affected lesion production and size; there were fewer lesions in mice, and the lesion size was greater in rats. The characteristics of the lesions produced in both species were similar to those described in studies by our research group and others, suggesting a common pathogenesis for the initiation and propagation of the lesions at the gross and microscopic levels.

I. INTRODUCTION

The effect of exposure timing quantities (pulse duration [PD], exposure duration [ED], total on-time, and pulse repetition frequency [PRF]) on the threshold for ultrasound-induced lung hemorrhage and on the size of the lesions at superthreshold levels has been examined to a very limited extent. Most of the studies that

have considered the role of timing quantities have focused on estimating threshold levels [1-4].

Child et al. [1] reported that the pressure threshold for lung hemorrhage at 3.7 MHz for a PD of 1 μ s was approximately twice that of a PD of 10 μ s (3.0-MPa and 1.5-MPa peak compressional pressure, respectively). The ED (180 s) and duty cycle (0.1%) were the same so therefore, PRF and number of pulses were different by a factor of 10. However, in the same article [1], the authors reported that at 1.2 MHz, PD of 10 μ s, and ED of 180 s, where they varied only the PRF, and hence the duty cycle or number of pulses, they obtained the same pressure threshold (0.7 MPa). **Frizzell et al. [4]** reported a decrease in threshold level with total on-time (and ED) in neonatal mice at 10°C at 1 MHz with a 10- μ s PD. The pressure thresholds they reported were approximately 0.37 MPa for 180-s ED (100-Hz PRF) and 1.5 MPa for 2.4-s ED (1-kHz PRF). **Raeman et al. [2]** reported that ED (3 min at 17-Hz PRF vs. 3 s at 1-kHz PRF) had a small effect on pressure threshold and extent of damage when the total on-time was held constant at 0.03 s for a PD of 10 μ s. Later, the same group [3] reported no statistically significant difference in the compressional pressure threshold (1.6 and 1.4 MPa) for ED of 20 and 180 s (10- μ s PD and 100-Hz PRF at 2.3 MHz), respectively.

The effect of timing quantities on superthreshold lesion development is more limited. **Raeman et al. [3]** reported differences in lesion size between 20- and 180-s ED, but data were not provided in the report. Earlier, **Raeman et al. [3]** reported that the lesion volume in mouse lung was greater for 3-min ED (100-Hz PRF) than for 0.3-min ED (1000-Hz PRF) suggesting a dependence of lesion growth on ED. However, the PRF was also different for the two exposures. There seems to be no published data evaluating independently the effects of PRF and ED.

Thus, the literature appears to show only a minor dependence of pressure threshold levels for ultrasound-

induced lung hemorrhage on timing quantities. There is a suggestion of an effect on the superthreshold lesion size, but little information is available. In this study, a more complete examination of the role of PRF and ED has been undertaken for superthreshold conditions in order to determine the effect of each variable and any interaction (number of pulses) between the two variables.

II. METHODS

Ultrasonic exposures used a focused 19-mm-diameter, lithium niobate ultrasonic transducer (Valpey Fisher, Hopkinton, MA) at a center frequency of 2.8 MHz. The *in situ* (at the pleural surface) peak rarefactional pressure was 12.3 MPa and the pulse duration was 1.42 μ s for all ultrasonically exposed animals.

The experimental protocol was approved by the campus' Laboratory Animal Care Advisory Committee and satisfied all campus and NIH rules for the humane use of laboratory animals. Animals were housed in an AAALAC-approved animal facility, placed in groups of three or four in polycarbonate cages with beta-chip bedding and wire bar lids, and provided food and water *ad libitum*.

Each experiment (mouse and rat) was a three-by-five factorial design with three exposure duration (ED) groups and five pulse repetition frequency (PRF) groups. A total of 165 six-to-seven-week-old 22.2 ± 0.2 -g female ICR mice (Harlan Sprague Dawley Laboratories, Indianapolis, IN) were randomly divided into 15 ultrasonically exposed groups (10 mice/group) and one sham group (15 mice). A total of 165 ten- to eleven-week-old 293 ± 28 -g female Sprague-Dawley rats (Harlan, Indianapolis, IN) were grouped identically to those animals used in the mouse experiment. Mice and rats were anesthetized with ketamine hydrochloride (87.0 mg/kg) and xylazine (13.0 mg/kg) administered intraperitoneally and exposed to pulsed ultrasound (ED: 5, 10 and 20 s; PRF: 25, 50, 100, 250 and 500 Hz).

Following exposure, animals were euthanized, and lungs were removed and fixed by immersion in 10% neutral-buffered formalin for a minimum of 24 hours. After fixation, the elliptical dimensions of each lung lesion at the visceral pleural surface were measured using a digital micrometer ("a" is the semi-major axis; "b" is the semi-minor axis). The lesion was then bisected and the lesion depth "d" within the pulmonary parenchyma was also measured. Each lesion surface

area (πab) and volume ($\pi abd/3$) were calculated.

The exposure conditions for each animal were revealed only after the final results were tabulated.

III. RESULTS AND DISCUSSION

As in our previous studies [5-6], none of the shams had lesions.

Proportion (percent) of animals with lesions

As PRF and/or ED increased, the percentage of animals with lesions approached 100% for both species (**Fig. 1**). Factorial analysis of variance showed that lesion production in mice and rats was related to PRF and ED, with the exception that PRF in rats was not significant. The PRF \times ED interaction was not significant for either species. Arithmetically, the PRF \times ED interaction is the total number of pulses, and a nonsignificant interaction suggests that the effects due to the number of pulses are additive from the individual observations (PRF and/or ED).

In the mouse, there was a clear increase in percentage of animals with lesions with both PRF and ED (**Fig. 1**). However, at the largest ED of 20 s, the percentage of animals with lesions had plateaued at 90% even at the low PRF of 50 Hz. Since there is an upper bound of 100% for percent of animals with lesions, this type of plateauing is to be expected at the higher exposures. For the rat, the proportion of animals with lesions was greater than for the mouse for similar exposure conditions (**Fig. 1**) despite the fact that the threshold for lesion formation has been shown to be essentially the same for the two species [6]. In addition, the plateauing of the percentage of animals with lesions with PRF seen in the mouse at an ED of 20 s is seen in the rat at essentially all EDs, but especially at EDs of 5 and 20 s. This finding is likely the reason that the results for the rat did not show a clear statistical significance with respect to PRF.

The dependence of lesion occurrence on PRF is supported by results of **Child et al. [1]** who showed a slightly larger proportion of mice with lesions for 100-Hz PRF as compared to 10-Hz PRF (1.2 MHz, 10- μ s PD and 180-s ED). Results of **Raeman et al. [3]** showed a greater proportion of mice with lesions for a 180-s ED as compared to a 20-s ED (2.3 MHz, 10- μ s PD and 100-Hz PRF) supporting the effect of ED seen in this study. Further support for the effect of ED was given by **Frizzell et al. [4]** who showed a greater percentage

of lesions in neonatal mouse lungs (1 MHz, 10- μ s PD) at 180-s ED (100-Hz PRF) than at 2.4-s ED (1-kHz PRF); however, the PRF was different for the two EDs.

Lesion depth, surface area and volume

The PRF, but not ED, significantly affected lesion depth in both species (**Fig 2**). Also, the PRFxED interaction for depth was not significant for either species. Both PRF and ED significantly affected lesion surface area in mice, while neither affected area in rats (**Fig 3**). Also, the PRFxED interaction for surface area was not significant for either species.

Lesion development in the mouse was clearly dependent upon PRF whereas surface area, but not lesion depth, was dependent upon ED (**Figs. 2-4**). In the rat, the picture is less clear regarding dependence on PRF since that was significant for lesion depth but not lesion area, although the rat results confirm the lack of dependence on ED for the range of EDs used in this study. The lack of dependence on ED seen in these results contrasts with results of **Raeman et al. [2]** who showed a significantly larger area of hemorrhage for a 3-min ED than for one 3-s ED or three 1-s EDs (1.2 MHz, 10- μ s PD); however, the PRF was different for the 3-min ED versus the other exposure durations and our results show a significant dependence on the PRF. In the same article, **Raeman et al. [2]** showed that the lesion volume was greater for 3-min ED than for 0.3-min ED, though the PRF was changed from 100 Hz to 1000 Hz, respectively, to maintain the same total on-time. It may also be significant that **Raeman et al. [2]** used a much larger range of ED than was used in this study; their longest ED was 180 s compared to our longest ED of 20 s.

Lesion volume was not an independent variable. Nevertheless, PRF and ED significantly affected lesion volume in mice, PRF but not ED significantly affected lesion volume in rats, and the PRFxED interaction for volume was not significant for either species (**Fig. 4**).

Species was not included in the randomized factorial design; each species was exposed separately for experimental convenience. As clearly seen in the figures, species significantly affected lesion occurrence (**Fig. 1**) and size (**Figs. 2-4**).

IV. SUMMARY

Prior to this study the effect of PRF and to a lesser degree ED on lesion progression in the lung was largely

unstudied. Our results have shown a clear effect of PRF and a less significant effect of ED, over the range of EDs used in this study. The fact that the PRF and ED both affect the percentage of animals with lesions suggests that these timing quantities should be considered within the definition of the MI, which applies to nonthermal mechanisms such as that operative in lung hemorrhage. The data from this study and others are not yet sufficient to completely define the timing effects (for example, the effect of pulse duration was not examined in this study), but they indicate that there should be further examination of timing quantities.

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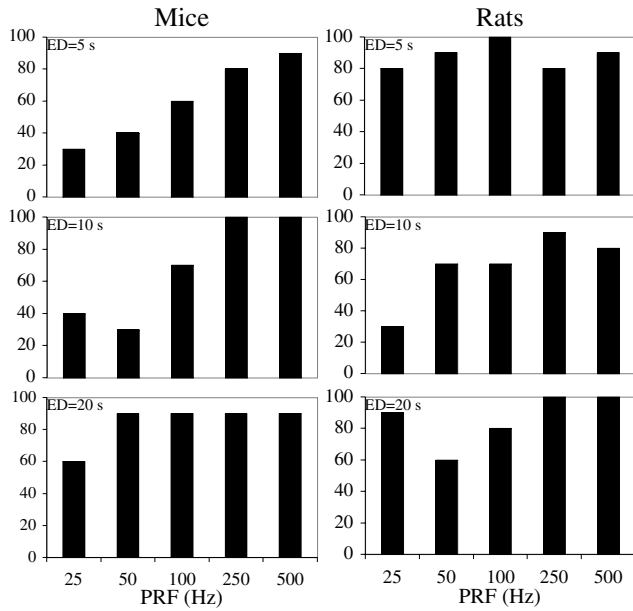


Figure 1: Percentage of lesions in mice (left panels) and rats (right panels) as a function of PRF for the three EDs.

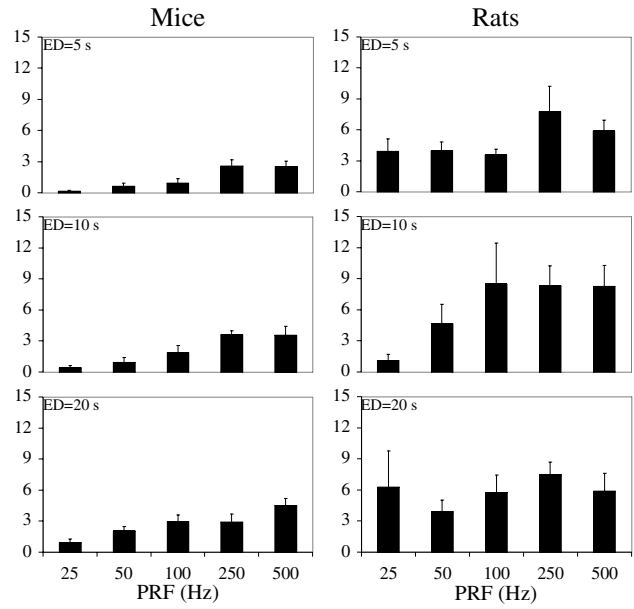


Figure 3: Mean lesion surface area (mm²) in mice (left panels) and rats (right panels) as a function of PRF for the three EDs. Error bars represent SEM.

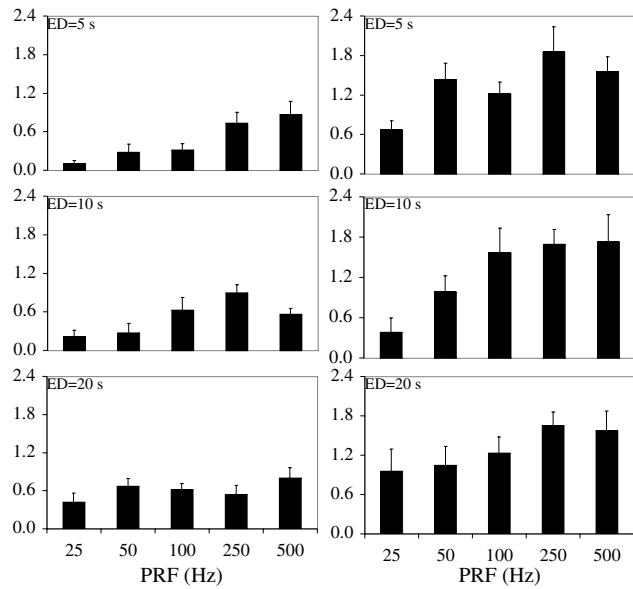


Figure 2: Mean lesion depth (mm) in mice (left panels) and rats (right panels) as a function of PRF for the three EDs. Error bars represent SEM.

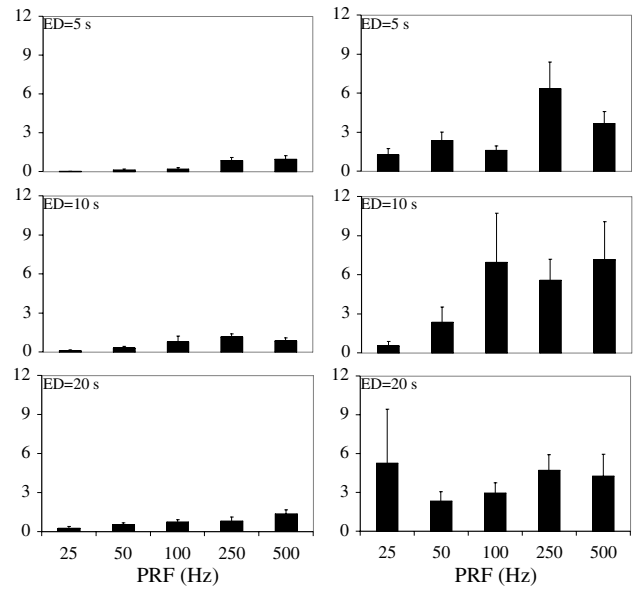


Figure 4: Mean lesion volume (mm³) in mice (left panels) and rats (right panels) as a function of PRF for the three EDs. Error bars represent SEM.