

Lesion Resolution Following Exposure of Rat Lung to Pulsed Ultrasound

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Abstract - Ultrasound has an exceptional safety record, but concerns have been raised by reports of clinical-level ultrasound-induced lung hemorrhage in mice, rats, rabbits, monkeys, and pigs. This study characterized the temporal reparative (healing) responses in lung following the induction of lesions by pulsed ultrasound (3.14 MHz, 1700-Hz PRF, 1.4- μ s pulse duration, 60-s exposure duration, *in situ* [at the pleural surface] peak rarefactional pressure of 17.0 MPa, and *in situ* peak compressional pressure of 39.7 MPa). Rats were anesthetized and the skin of the left thorax clipped and depilated. They were placed in right lateral recumbency and two black dots were positioned on the skin over the left intercostal spaces at the sixth and the ninth ribs. The transducer was aligned with the first black dot and the rat was exposed. The process was repeated over the second dot. Following exposure, lung lesions were evaluated at 0, 1, 2, 5, 7, 9, 12, 14, and 16 days post exposure (dpe). Lungs were scored for the presence of lesions, recorded digitally, and fixed in 10% formalin. After fixation, the dimensions of each lesion at the visceral pleural surface were measured. The lesions were bisected and the depth measured. Each half of the bisected lesion was processed and evaluated microscopically. Lesions at 0 dpe were large bright red ellipses of hemorrhage that formed under the visceral pleura; the visceral pleura was intact. By 2 dpe, the lesions were smaller in size and dark red to red-black. At 5 and 7 dpe, the lesions were smaller in size and gray to yellow-brown. Between 9 and 16 dpe, lesions were yellow-brown and the size and the quantity of pigment was substantially decreased. By 16 dpe the lung area with lesions had returned to a near normal appearance. The temporal changes were indicative of degradation of erythrocytes through processing and removal of hemoglobin and iron pigments. Microscopic lesions par-

alleled the gross lesions and reparative responses resulted in minimal alteration of lung structure. The reparative response in lung was analogous to reparative responses in soft tissues associated with bruising, but also had a proliferative phase characterized by focal hyperplasia of spindle cells whose phenotypes need to be determined.

I. INTRODUCTION

Although pulsed ultrasound is one of the safest imaging modalities, concerns have been raised and addressed by members of the bioeffects research community [1]. These concerns resulted from publications describing lung hemorrhage induced in laboratory animals at exposure conditions similar to those used for scanning in humans [2-14]. Although the pathogenesis of ultrasound-induced lung hemorrhage is unknown, recent studies have determined that inertial cavitation is not involved in tissue injury [15]. Mechanical phenomena, such as those associated with radiation force effects, are more likely involved in ultrasound-induced lung hemorrhage. The purpose of this study was to macroscopically and microscopically characterize the reparative responses of rat lung following superthreshold exposure to pulsed ultrasound.

II. METHODS

Ultrasonic exposures used a focused 51-mm-diameter lithium niobate ultrasonic transducer (Valpey Fisher, Hopkinton, MA) at a center frequency of 3.14 MHz, pulse repetition frequency of 1700-Hz, pulse duration of 1.4- μ s, and an exposure duration of 60 seconds. The *in situ* peak rarefactional pressure at the pleural surface was 17.0 MPa and the *in situ* peak compress-

sional pressure was 39.7 MPa. Twenty seven 10- to 11-week-old female Sprague-Dawley rats (Harlan, Indianapolis, IN) were anesthetized with ketamine hydrochloride (87.0 mg/kg) and xylazine (13.0 mg/kg) administered intraperitoneally and exposed to pulsed ultrasound in right lateral recumbancy (Figure 1).

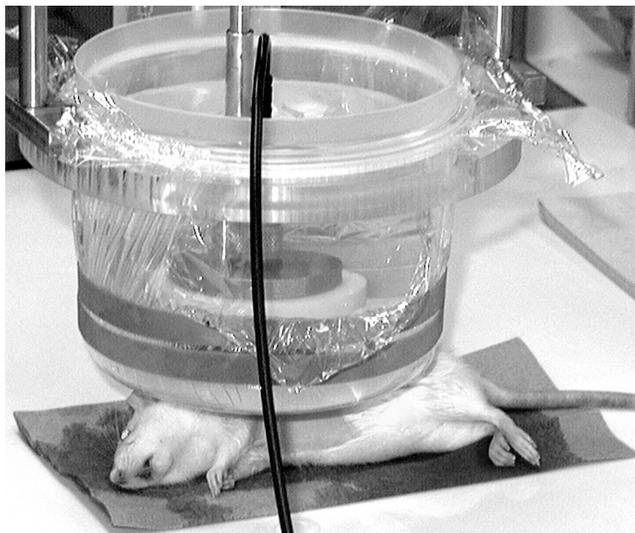


Figure 1: Exposure standoff apparatus and transducer setup used to deliver superthreshold pulsed ultrasound to the lateral surface of the left lung.

The skin of the left thorax was clipped and depilated. Rats were placed in right lateral recumbancy and two black dots were positioned on the skin over the left intercostal spaces at the sixth and the ninth ribs. The transducer was aligned with the first black dot and the rat was exposed. The process was repeated over the second dot. Following exposure, lung lesions were evaluated at 0, 1, 2, 5, 7, 9, 12, 14, and 16 days post exposure (dpe). Three rats were killed at each time point post exposure by cervical dislocation while under anesthesia. The left lung was evaluated for gross lesions, fixed by immersion in 10% neutral-buffered formalin, embedded in paraffin, sectioned at 5 μm , stained with H&E, and evaluated microscopically.

III. RESULTS AND DISCUSSION

Macroscopic Lesions

At 0 dpe, lesions were red elliptical areas of hemorrhage under the visceral pleural surface (Figure 2). The hemorrhage extended into subjacent parenchyma to form a conical shape. The base of the cone was at the pleural surface and the apex was within the lung

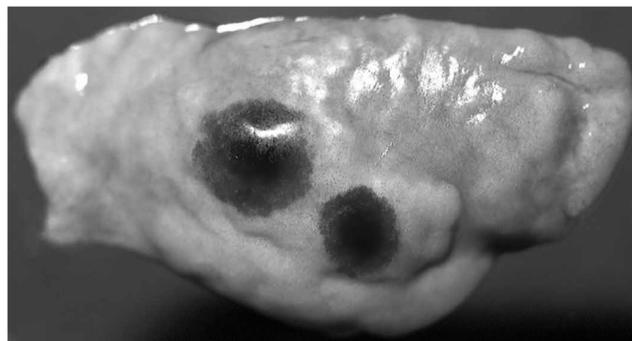


Figure 2: Red elliptical areas of hemorrhage under the visceral pleural surface at 0 dpe following superthreshold exposure to pulsed ultrasound.

parenchyma at the deepest extent of the lesion. Lesions retained a similar overall shape but gradually decreased in surface area, depth, and volume (Figure 3).

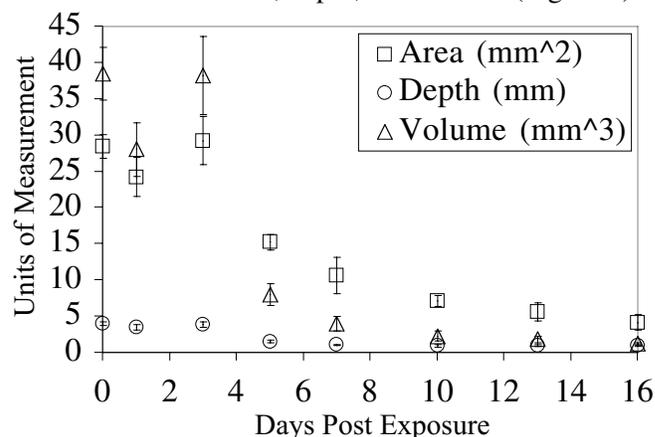


Figure 3: Temporal changes in lesion surface area, depth, and volume following superthreshold exposure to pulsed ultrasound.

There was an overall color change that progressed from red to dark red to light yellow-brown (Figure 4). From 3 through 16 dpe, the surface of the lesions was raised

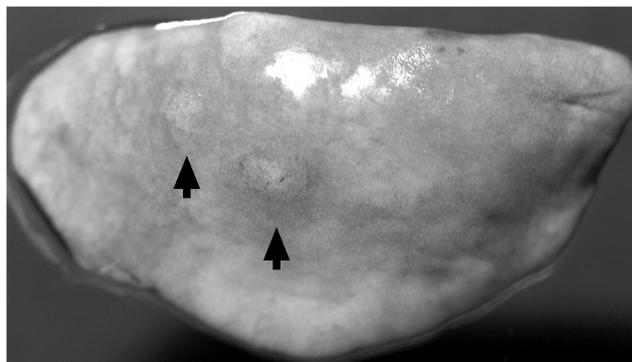


Figure 4: Light yellow-brown areas (arrows) of hemosiderin pigment under the visceral pleural surface at 16 dpe following superthreshold exposure to pulsed ultrasound.

and lighter in color than the surrounding injured area.

Microscopic Lesions

Lesions at 0 dpe were characterized by areas of acute alveolar hemorrhage under the visceral pleural surface without pleural or septal injury. From 1 through 10 dpe, lesions showed erythrocyte degradation, accumulation of hemoglobin crystals, and the production of hemosiderin pigment. Beginning at 1 and continuing through 16 dpe, a proliferative cellular reparative response consisting of the proliferation of large numbers of epithelial and mesenchymal cells in the exposed area that altered the normal alveolar architecture was also observed (Figures 5-6).

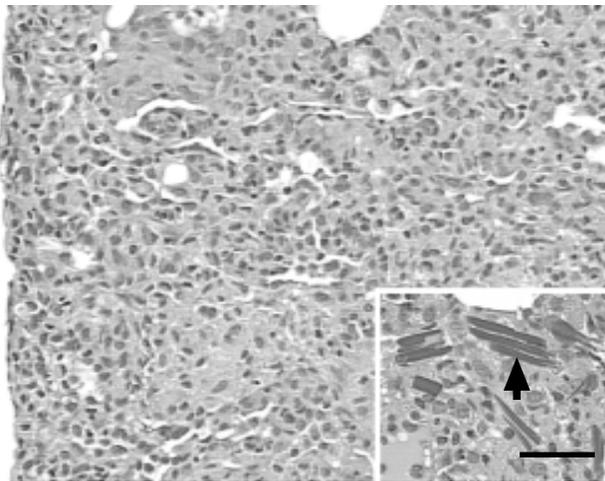


Figure 5: Proliferating cells in lung at 5 dpe following superthreshold exposure with pulsed ultrasound. Alveoli are not apparent and hemorrhage is resolved. Inset: hemoglobin crystals have formed (arrow). H&E stain, Bar = 40 μ m.

The phenotype(s) of these cells could not be adequately determined by light microscopy, but likely include type II alveolar epithelial cells, alveolar macrophages, smooth muscle cells of vascular origin, and fibroblasts. This response diminished in severity with dpe. Ultrasound-induced lung injury resulted in severe alveolar hemorrhage and a vigorous reparative response. Alveolar macrophages (blood monocytes) would be expected to play an important role in removing and degrading erythrocytes, processing hemoglobin pigment, and removing hemosiderin pigment from lung. The proliferation of probable type II alveolar epithelial cells, fibroblasts, and other unidentified spindloid cell types was unanticipated, but type II alveolar epithelial cells are the cells in lung that proliferate following other types of toxic, metabolic, and infectious injury. The tissue

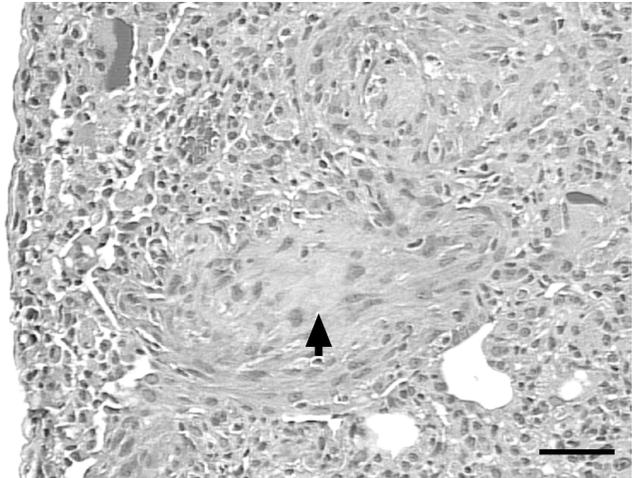


Figure 6: Proliferating spindloid cells (arrow) in lung at 5 dpe following superthreshold exposure with pulsed ultrasound. These cells may be type II alveolar epithelial cells, alveolar macrophages, smooth muscle cells of vascular origin, or fibroblasts. H&E stain, Bar = 40 μ m.

and cellular characteristics of this proliferative response may be related to cytokines or other growth factors associated with the elicited eosinophilic leukocyte inflammatory response. The role of ultrasound and its interaction with resident and recruited inflammatory cells in potentiating inflammatory and proliferative reparative responses warrants further investigation. Microscopically, injured lung returned to near normal by 16 dpe but had small foci of fibrosis and hemosiderosis (Figure 7).

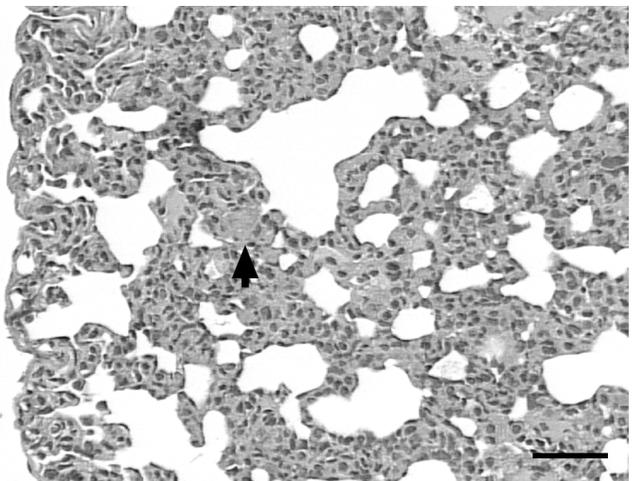


Figure 7: Reappearance of alveoli at 16 dpe following superthreshold exposure with pulsed ultrasound. Subpleural and septal areas contain small foci of fibrosis and hemosiderosis (arrow). H&E stain, Bar = 40 μ m.

IV. SUMMARY

Ultrasound induced lung injury resulted in severe alveolar hemorrhage and a vigorous reparative response. The reparative response that ensued under the superthreshold exposure conditions described in this study resulted in no substantive long term residual effects that would be detrimental to normal alveolar function and air exchange. Macroscopically and microscopically, injured lung returned to near normal by 16 dpe but had small foci of fibrosis and hemosiderosis. Based on morphologic analysis, it was likely that lung function was also normal.

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