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●*Original Contribution*

DAMAGE TO MURINE KIDNEY AND INTESTINE FROM EXPOSURE TO THE FIELDS OF A PIEZOELECTRIC LITHOTRIPTER

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Abstract—Earlier studies, in which murine kidneys were exposed to spherically diverging, spark-generated shock waves, demonstrated extensive hemorrhage in the interior of the organ at peak positive pressures somewhat less than 10 MPa. With comparable pulse numbers, this investigation, using the focal fields of a piezoelectric lithotripter, found no damage to murine kidneys at peak positive pressures as high as 40 MPa. Comparison of these cases and earlier bioeffects studies using pulsed, focused ultrasound leads to the conclusion that damage to murine kidneys is not simply correlated with peak positive pressure or peak negative pressure, nor is spectral content of the wave able to explain the striking differences in damage from these sources. With 200 individual shock waves from the piezoelectric lithotripter applied ventrally, 20–30% of the animals suffered *superficial* kidney damage (bleeding into the capsule), but the same exposure conditions produced severe intestinal hemorrhage in more than 80% of the animals.

Key words: **Piezoelectric lithotripter, Kidney hemorrhage.**

INTRODUCTION

Delius and co-workers (1988 a and b) were among the first to describe damage to kidney tissues associated with lithotripter treatment of kidney stones. Our attempts to determine thresholds for tissue damage from shock wave exposure have produced strikingly different biological results depending upon the nature of the source. Just 10 spark-generated, spherically diverging double shocks from a Wolf endoscopic lithotripter at peak positive pressures as low as 3 MPa resulted in extravasation of erythrocytes in murine kidneys (Mayer et al. 1990). In these tests, the medulla of the kidney was found to be more sensitive to damage than the overlying cortex, muscle and skin. On the other hand, murine kidneys exposed to focused, pulsed ultrasound with peak positive pressures approaching 10 MPa, where nonlinear propagation transformed each cycle of the incident wave into an acoustic shock, showed no significant extravasation even though the total numbers of individual shocks in certain exposures exceeded ten million (Carstensen et al. 1990). In a continuing search for the acoustic characteristics of the exposures that correlate with tissue damage, we report here on studies performed with an experimental piezoelectric lithotripter.

EXPERIMENTAL METHODS

The acoustic source used for the exposures was a custom piezoelectric lithotripter constructed originally by Ford Laboratories, Inc. (Dublin, CA) and remodeled by Specialty Engineering Associates (Milpitas, CA). The construction of the device is described in detail in an earlier paper (Dalecki et al. 1991). An aluminum lens, approximately 50 cm in diameter and with a focal length of 54 cm, forms the bottom of the exposure tank. A bank of piezoelectric elements fixed to the rear of the lens is charged to approximately 8 kV and discharged to produce the shock wave. The laboratory lithotripter differs from similar commercial lithotripters primarily by having an open coupling medium that provides greater flexibility in the placement of small animals for exposure. The focal wave form, as measured by an Imotec Type 80-0.5-4.0 PVDF needle hydrophone (Imotec Gmbh, Würselen, Germany) is shown in Fig. 1a. For the animal exposures in this investigation, the water level in the tank was adjusted to correspond to the axial midpoint of the focal region. Figure 1a is the direct wave and does not include possible reflections that are discussed below.

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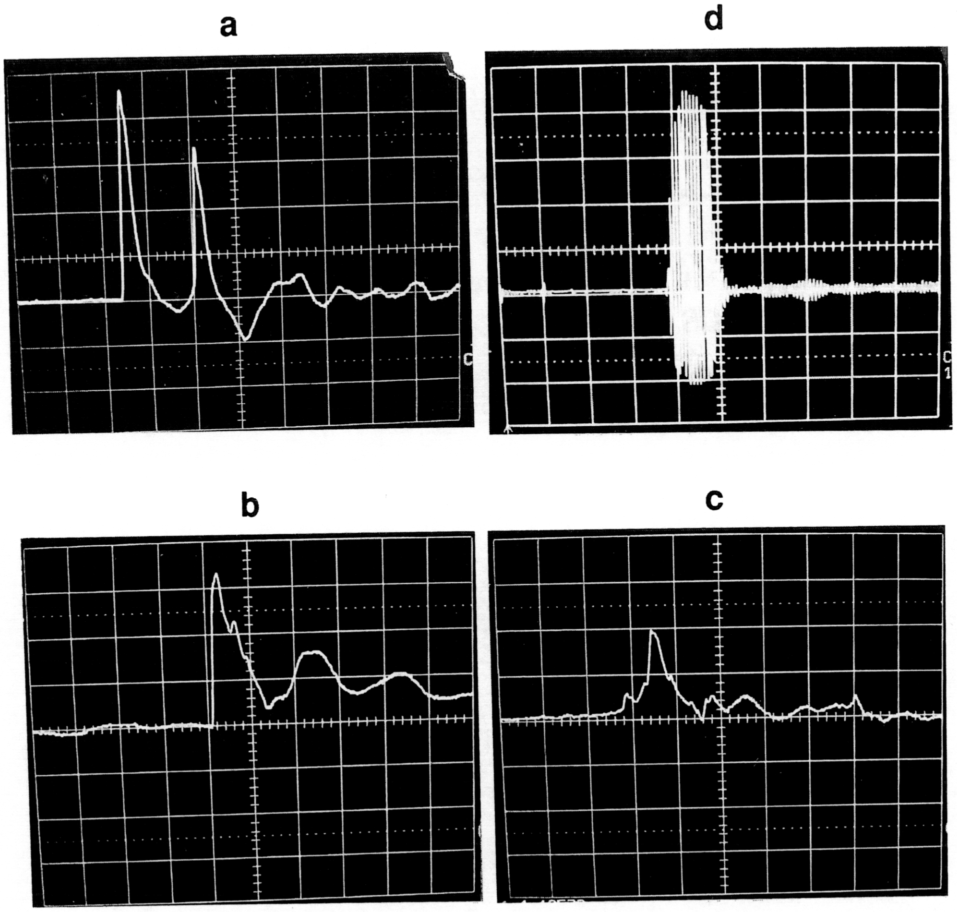

Fig. 1. Waveforms used in our studies of the effects of shock waves on the murine kidney. All fields were measured with an Imotec hydrophone in water. (a) The focal pressure for the piezoelectric lithotripter used in this study, $p_+ \sim 40$ MPa, 1 μs/div. (b) and (c) The pressures generated by the Wolf endoscopic electrohydraulic lithotripter, 1 μs/div (Mayer et al. 1991). The first shock, shown in Fig. 1b, ($p_+ \sim 4$ MPa) is generated directly by ignition of the spark, and the second, shown in Fig. 1c, ($p_+ \sim 2$ MPa) occurs approximately 1 ms later at the rebound of the plasma bubble. With 10 of these double shocks, the threshold for damage to the kidney was approximately 3 MPa (Mayer et al. 1990). Negative pressures were too small to be measured. (d) Focal pressures for the 1.2 MHz pulsed ultrasound source from Carstensen et al. (1990), where no histological evidence of damage to the kidney was seen. Here, the peak positive pressure is 12 MPa; maximum negative pressure, 4 MPa; pulse length, 10 μs (10 μs/div); pulse repetition frequency, 2000 Hz; exposure duration, 3 min.

C3H mice were anesthetized with Ketamine (200 mg/kg) and Rompun (10 mg/kg) (intraperitoneally, 0.2 mL for a 25 g mouse), their ventral and dorsal surfaces were shaved and depilated with Neet®. They were positioned horizontally by limb restraint on an animal holder. The holder, in turn, was supported on a three-way positioner that allowed stable, precise location of the mouse with respect to the source. The head of the animal was supported with its nose above the water level. The position of the kidney was determined by palpation and its center marked by a spot of ink on the upper surface of the animal. The coordinates of the axis of the focal field were determined with the hydrophone, and these measurements were used to transfer the center of the kidney to the desired field position. The positioning technique was demonstrated to be accurate within ±2 mm by placing a mouse with its ventral surface toward the source very near the surface of the water, and observing that the acoustic fountain occurred directly above the center of the kidney. When the mice were oriented with their ventral surfaces facing the source, they were supported on a sheet of 20 μm thick, plastic film (Reynolds Plastic Wrap®) tightly stretched across the animal holder. Contact with the film was made with ultrasound coupling gel. After exposure, the kidneys were removed, the animals were euthanized by cervical dislocation and hemorrhage in other organs, particularly the lung and intestine, was noted. The kidneys were fixed in 3.7% formaldehyde for 24 h, and subsequently sectioned and inspected visually for presence or absence of hemorrhage.

To estimate the pressures at the kidney when the ventral surface of the animal faced the source, the insertion loss of a mouse mounted as described above was measured. Two three-way positioners were used, one for the mouse and one for the hydrophone. The hydrophone was placed at the focus of the lithotripter and the mouse was moved in and out of the field below the hydrophone. The total energy in the shock wave was measured by integrating the square of the voltage from the Imotec hydrophone over the period of the pulse (3.5 μs) using the internal software of a LeCroy Model 9400 (Chestnut Ridge, NY) digital oscilloscope. The ratio of the shock energy with and without a mouse in the sound path was determined. The beam pattern was approximately the same with and without the mouse in place, indicating that scattering and refraction were not the dominant factors in the attenuation.

The frequency spectra of the wave forms of the focused piezoelectric lithotripter fields used in this investigation, the spherically diverging electrohydraulically generated shock waves used in Mayer et al. (1990) and the pulsed ultrasound used in Carstensen et al. (1990) were determined by use of the Fourier transform software internal to the LeCroy Model 9400 Oscilloscope. The LeCroy computed the power spectrum relative to a sine wave with an amplitude of 0.316 V in each case. Rectangular windows of 10 μs were used in analyzing lithotripter wave forms and 100 μs for pulsed ultrasound, and all were sampled at 12.5 MHz. Because the Imotec PVDF needle hydrophone was used for the primary comparison of spectra of the acoustic fields, its calibration is common to each of the spectra. Spot comparisons of the Imotec with a Marconi bilaminar hydrophone indicated that its response is uniform ±3 dB from 0.5 to 10 MHz. To test for the possibility that there might be significant differences in the sources at very low frequencies, similar observations were made using a Brüel & Kjaer (B & K, Copenhagen, Denmark) No. 8103 hydrophone, which has a uniform response ± 2 dB from 0.1 Hz to 200 kHz.

EXPERIMENTAL RESULTS

Field levels

Figure 1a shows the focal shock pressures from our piezoelectric lithotripter measured in water. The peak positive and negative pressures at the surface of the animal were approximately 40 MPa and −9 MPa. As shown in Fig. 1a, the rise time of the shock wave at the focus is less than 200 ns, an estimate limited by the band width of the hydrophone (~15 MHz). The −3 dB width of the focus is approximately 4 mm. The −3 dB "axial length" of the focus is approximately 40 mm.

Figures 1b, 1c and 1d show the waveforms of the shock waves used in our two previous studies of extravasation in mouse kidney. Figure 2 gives the corresponding power spectra of the waves used in those studies. In addition to frequency and amplitude differences, of course, the exposures differed physically in the relative amplitudes of the positive and negative phases of the waves. Two of the fields are focused and the third is spherically diverging. Furthermore, with the electrohydraulic source (Figs. 1b and 1c), each exposure consisted of a wave launched upon ignition of the spark followed approximately 1 ms later by a second, largely positive pressure pulse that had an amplitude and energy approximately equal to the initial shock created by the spark.

Only at the lowest frequencies (< 200 kHz) do the spectra shown in Fig. 2 for the piezoelectric and electrohydraulic lithotripter fields approach each other. For a more direct and reliable comparison between these two sources at low frequencies, their outputs were measured with a No. 8103 B & K hydrophone. In the frequency range up to 200 kHz, the focal pressures of the piezoelectric lithotripter as used in these studies (40 MPa peak positive pressure) were approximately 15 dB greater than those of the electrohydraulic lithotripter measured at a point in the field where its temporal peak positive pressure was 3 MPa.

The insertion loss of the mouse varied from subject to subject and position to position for a single animal. In one animal, for example, the attenuation was 4 dB with the beam positioned through one kidney and 7 dB through the other kidney. On average, the levels at the kidney when exposed through the ventral surface were approximately 6 dB less than the incident pressures.

Pathology

Our earlier study showed that 10 double pulses from the Wolf lithotripter (Figs. 1b and 1c) produced damage to the interior of the kidney at peak positive pressures as low as 3 MPa (Mayer et al. 1990). Exploratory observations suggested that the threshold for kidney hemorrhage with the piezoelectric lithotripter was much greater. For a direct comparison of the kidney pathology produced by the two lithotripters, a set of eight mice were exposed to 20 shock waves at the focus of the piezoelectric lithotripter, with their dorsal surfaces facing the source. Twenty shocks were used with the piezoelectric lithotripter to compare with 10 spark ignitions, because each spark produced two high amplitude pressure waves, one at ignition and the second at rebound of the plasma bubble. Because only approximately 1 mm of skin and muscle overlies the kidney, the proximal side of the kidney experienced shock waves very similar to those shown in Fig. 1a.

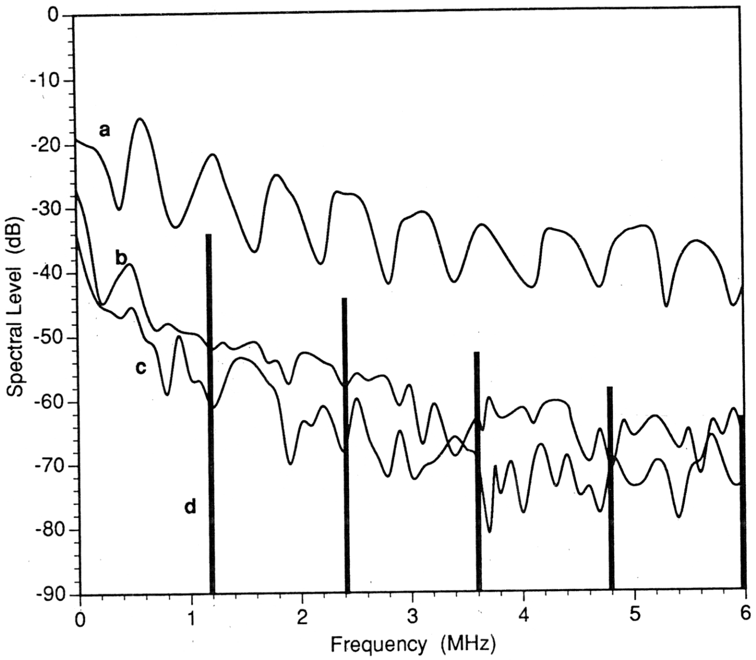

Fig. 2. Frequency spectra of the sources shown in Fig. 1. The fields were measured with an Imotec PVDF hydrophone and analyzed by the Fourier transform software of the LeCroy Oscilloscope. (a) Focal field of the piezoelectric lithotripter ($p_c \sim 40$ MPa in water). (b) Initial shock of the Wolf lithotripter ($p_+ \sim 4$ MPa in water). (c) Shock from rebound of the primary bubble of the Wolf lithotripter ($p_+ \sim 2$ MPa in water). (d) Focal field of a 1.2 MHz pulsed ultrasound source ($p_+ \sim 12$ MPa in water). Note that the spectrum in (d) is limited to narrow bands at the fundamental, 1.2 MHz and harmonics. The long tone burst produces the narrow bands, and nonlinear propagation produces the harmonic components.

One of the eight exposed kidneys had a superficial petechia that was barely visible to the naked eye. Otherwise, there was no evidence of damage in spite of the fact that the peak positive pressure at the proximal surface of the kidneys approached 40 MPa. Whereas twenty pulses with the piezoelectric lithotripter at 40 MPa peak positive pressure caused essentially no damage to the kidney, we showed in an earlier study (Mayer et al. 1990) that ten, 10 MPa double shock waves from an endoscopic electrohydraulic lithotripter (Wolf) produced massive damage throughout the organ, and there was significantly greater damage to the kidney with the endoscopic electrohydraulic lithotripter at a peak positive pressure of 3 MPa than with the piezoelectric lithotripter at 40 MPa.

Another series of animals was exposed at the focus with their ventral surfaces facing the source. The target site in each exposure was the left kidney. The pressures were smaller at the kidney because of attenuation of the wave in passing through the bowel, but the shock wave number for each exposure was increased to 200. With the dorsal surface exposed to the air, there was an increased possibility that large negative pressures might be produced in some regions of the kidney by reflection of the shock wave from the pressure release tissue-air interface. In this part of the investigation, 19 mice were exposed with air above the shaved and depilated backs of the animals (Condition A), and, for comparison, an additional 10 mice were exposed with an absorber above the kidney (Condition B). The absorber consisted of a water-filled 3.8 cm diameter, plastic, cylindrical tube 10.5 cm in length, with a 2.5 cm thick natural rubber upper termination. The autopsy results are summarized in Table 1. The kidneys of animals in both groups showed remarkably little evi-

Table 1. Summary of the autopsy results for mice exposed to 200 lithotripter shocks.

Tissue	Negative reflection Condition A (n = 19)	No reflection Condition B (n = 10)
Kidney	0.2	0.3
Lung	1.0	1.0
Intestine	0.8	1.0
Ventral skin	0.7	0.8

Peak positive pressure at the ventral surface of the animal was ~40 MPa. Estimated peak positive pressure at the dorsal surface of the kidney was approximately 20 MPa. The backs of the first group of animals were in contact with air. For the second group of animals, reflections were minimized by a water/rubber interface. The table indicates the fraction of the animals in each group that were found to have hemorrhages.

dence of tissue damage. Where bleeding occurred, it involved collection of only a small amount of blood under the capsule. There was no indication of damage to the medulla or the cortex of the kidney of any animal in the study.

All animals in the study showed at least some degree of lung hemorrhage. Hemorrhage in the intestine was very common (Table 1). There was widespread bleeding in the intestinal wall and into the lumen but no blood was seen in the peritoneal cavity. Skin petechiae on the ventral surface also were common. Overall, there were no clear differences between the effects with animals backed by air and those backed by an absorber.

DISCUSSION

In the three investigations of the effects of shock waves on murine kidneys that we have conducted thus far, the pathology has been strikingly different. With spherically diverging, spark-generated shock waves, the medulla was the most sensitive structure (Mayer et al. 1990). With pulsed ultrasound, we were unable to identify any damage to the kidney at the highest pressure levels that we could produce (+ 10–20 MPa). The piezoelectric lithotripter permitted us to go to higher peak pressures. When only 20 shock waves were used, as in the electrohydraulic lithotripter study, essentially no damage was observed in the kidneys in spite of the fact that the pressure levels were at least an order of magnitude greater than those that produce significant kidney hemorrhaging with our spherically diverging electrohydraulic source. With 200 shock waves and peak positive pressures of the order of 20 MPa, we found only a small amount of bleeding into the capsule of one quarter of the kidneys, but no apparent damage to the cortex or medulla, the substructure most sensitive to the effects of the electrohydraulic source.

It has been suggested that lithotripters are more destructive to tissues than pulsed ultrasound at equivalent pressure levels because their fields are predominantly lower in frequency. Although it is true that, above 1 MHz, the threshold for cavitation increases with frequency, it is not clear that that trend is valid much below 1 MHz (Flynn 1982). We compared the frequency spectra of the three sources that we have used in studies of kidney damage (Fig. 2). Each source was observed through the window of sensitivity of the Imotec hydrophone for comparison at high frequencies, and through the B & K hydrophone for very low frequencies. (Of course, the spectrum of the pulsed ultrasound fields (Fig. 1d) consists primarily of the carrier frequency and its harmonics that are generated by nonlinear propagation. No histologically detectable effect was observed after exposure of the kidneys to more than 100,000 of the pulses shown in Fig. 1d.) Our question is whether the striking differences in the effects of the two lithotripter exposures can be related to differences in the frequency content of their waves. The amplitude of the spectrum of the piezoelectric lithotripter used in this study is greater at all frequencies than that of the more effective, 3 MPa, spherically diverging, spark-generated shock wave. This is true even at the low frequencies measured with the B & K hydrophone. It is true even after taking into consideration the 6 dB attenuation of the piezoelectric lithotripter's field in passing through the animal in the second series of tests. When equal numbers of shocks were used, the threshold for hemorrhage expressed in terms of low frequency pressure levels is greater for the piezoelectric lithotripter, and the damage produced at comparable low frequency pressures is much less than it is for the spark-generated shock wave. Two hundred of the shocks from the piezoelectric lithotripter (Curve 1a, but attenuated 6 dB by passing through the bowel) produced superficial hemorrhaging in only 20 to 30% of the exposed kidneys. Thus, it appears that the frequency characteristics of lithotripters alone are not an adequate explanation for their effects on tissues such as kidney.

With the piezoelectric lithotripter, the organs known to contain air bubbles were most sensitive to damage. The lungs of every animal hemorrhaged. The lungs were not on the axis of the sound field in these experiments. We estimate from the beam pattern of the lithotripter that the maximum positive pressures experienced by the lung were approximately 10 MPa. Because this is somewhat greater than the thresholds that have been observed for exposure of the lung to spherically diverging shock waves (Hartman et al. 1990) and pulsed ultrasound (Child et al. 1990), it is not remarkable that the lungs of the mice in this study were damaged.

The observed skin lesions apparently arise from a physically different exposure environment than the other organs. The highly localized hemorrhages that we observed on the ventral surface of the animals very probably result from cavitation in the coupling medium very near the surface of the body. This hypothesis is supported by the observation that these petechiae could be eliminated by covering the skin of the mice with a 2 mm thick layer of vacuum grease.

The high incidence of intestinal hemorrhage was a surprise to us. Earlier, Chaussy et al. (1986) had reported intestinal hemorrhage in rats exposed to the Dornier lithotripter. In their experiments, the intestine was eventrated and embedded in vaseline for coupling but otherwise was backed by air. But, even though we made no systematic study of the intestine in the earlier investigation of kidney hemorrhage with the endoscopic, electrohydraulic lithotripter, our records indicate that it was very rare. Paradoxically, the endoscopic, electrohydraulic lithotripter caused injury throughout the kidney and little intestinal hemorrhage; but, the piezoelectric lithotripter produced only occasional superficial kidney damage but a high incidence of intestinal hemorrhage. It is tempting to attribute the large scale hemorrhage in the intestine with the piezoelectric lithotripter to the presence of gas bodies. Lehmann and Herrick (1953) reported petechiae in the peritoneal surfaces of mice as the result of the use of exposures characteristic of ultrasonic diathermy (3 W cm^{-2}, CW) and presented substantial evidence that the responsible mechanism was cavitation. Cowden and Abell (1963) also reported intestinal hemorrhage with CW ultrasound at similar intensities. Miller and Thomas (1994) confirmed these observations of intestinal hemorrhage, but concluded from their study that heat is the predominant mechanism of injury. Pulsed ultrasound exposures used in diagnosis are in a sense intermediate between the low level, CW exposures used by Lehmann and Herrick and those used by Miller and Thomas, on the one hand, and the high level, short duration lithotripter fields used here. Clearly, the mechanisms of damage by lithotripter fields are nonthermal. Therefore, it is not possible to rule out intestinal hemorrhage in diagnostic examinations simply because the thermal index is small. Because the intestine is commonly exposed during many diagnostic examinations, the observations reported here suggest that it will be important to determine thresholds for damage to the intestine from exposures to pulsed ultrasound.

Our experimental piezoelectric lithotripter, with maximum pressure in water of ~40 MPa, has a smaller output than typical clinical piezoelectric lithotripters. However, the tissue attenuation in our studies with mice is so small that the pressure levels in the kidney used in our study are comparable to those experienced in this organ during clinical lithotripsy procedures. The relative levels of tissue injury seen for different lithotripters is a factor to be considered in choice of shock wave sources for the treatment of kidney stones.

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