

Threshold Dosages for Damage to Mammalian Liver by High Intensity Focused Ultrasound

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Abstract—The threshold dosages for high intensity focal lesion production were determined at 3 MHz in the cat liver for exposure durations covering the range 0.003–35 s. The liver threshold was found to parallel that for the brain over the range of exposure durations 0.1 to 10 s, but to be more than twice the intensity level, approximately following the relation $I T^{0.5} = 460 \text{ W} \cdot \text{cm}^2 \cdot \text{s}^{0.5}$ where I is the peak intensity and T is the exposure duration. At shorter exposure durations the threshold curve deviates from this relation, probably due to a change to a transient cavitation mechanism of damage.

INTRODUCTION

IN RECENT YEARS the threshold dosages required for the production of focal lesions in mammalian brain [1]–[4] and other tissues [5] have been reported. Chan and Frizzell [6] extended the investigations of the liver to intensities above those previously reported for this tissue, demonstrating that the levels for damage to liver were more than twice those for brain, over a broad range of exposure durations.

Much information has been gathered from the results of these studies concerning the levels for, and mechanisms for, damage from ultrasound. More specifically, it has been demonstrated by several studies that the mechanism for damage from focused ultrasound at low intensities (below about $500 \text{ W}/\text{cm}^2$) is thermal. At high intensities (above $2\text{--}3 \text{ kW}/\text{cm}^2$) the evidence strongly suggests a cavitation mechanism. Lesions in both the brain and liver in this high intensity range have irregular boundaries and exhibit much more severe damage to the tissue than seen at lower intensities. In the brain these lesions occur preferentially near interfaces, not necessarily at the focus of the sound field, and they occur immediately after irradiation whereas those at low intensities require time to develop. Herein is reported more data providing further evidence for different mechanisms of damage at high and low intensities.

METHODS

The methods employed to determine the threshold levels for damage to liver from focused ultrasound are described in part elsewhere [6] but are provided here for

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completeness. Adult cats were anesthetized with intraperitoneally administered Diabuto™ at a dose of $0.55 \text{ ml}/\text{kg}$ and the liver exposed via a midline incision. A pan with an opening in the bottom was placed above the liver and a watertight seal formed between the abdominal skin and the opening using a wire tourniquet. Ink marks were placed on the liver, accessible through the opening in the pan, at the boundary of the area containing the irradiation sites. These marks served as guides for irradiation at a number of sites, usually nine, separated by approximately 1 cm. The right median lobe of the liver was used for the irradiations except for occasional animals in which the presentation of the liver was such that it was much more convenient to use the left lateral lobe. After marking, the pan was filled with degassed mammalian Ringer's solution preheated to 37°C and maintained at that temperature by circulating thermally regulated water through a heating coil mounted in the pan. The Ringer's solution provided an osmotically compatible, temperature regulated, coupling medium and an acoustic match to the back side of the liver to ensure no localized heating at that interface [5], [7].

The transducer had been used for previous lesioning studies of the brain [8] and consisted of a 1-MHz fundamental quartz disc that was coupled to a stepped plastic lens. The transducer aperture was 10 cm, and the focal length 13.7 cm resulting in lengths between half power levels at the focal region of 0.95 mm transverse to the axis and 5.8 mm along the axis when driven at 3 MHz. The transducer was rigidly mounted on a three dimensional positioning system and had an attached pointer system adjusted to place the center of the focal region approximately 3 mm below the liver surface when the pointer was placed in contact with the surface. The pointer was retracted and removed from the sound field, and any bubbles that may have collected at the transducer face were removed by suction prior to excitation of the transducer. Motion of the liver with respect to the sound beam was eliminated by giving the cat a muscle relaxant (Pavulon™ administered intravenously at a dose of $0.06 \text{ ml}/\text{kg}$ with an equal dose of atropine) and placing it on a respirator. The respirator was stopped during each irradiation to eliminate movement of the liver.

Permanent thread markers of 5 - 0 surgical silk were applied over the ink marks at the boundary of the irradiated region upon completion of irradiation at all sites. For

animals sacrificed at 1 h after irradiation, the surgical incision was clamped whereas those sacrificed at 24 h were closed with surgical silk and allowed to recover from the muscle relaxant and the anesthetic. At sacrifice the animal was administered an overdose of the anesthetic, cannulated through the left ventricle, flushed with 900 ml of buffered saline, and perfused with a 10 percent formalin, 90 percent buffered saline fixative for approximately 40 min. The region of the liver lobe containing the irradiation sites was removed, and pins were inserted near the edges to eliminate any ambiguity as to the orientation of the excised sample upon later examination of microscope slides. The excised sample was dehydrated, embedded, and serially sectioned (usually at a 10- μm thickness); and every tenth section was mounted and stained with Hematoxylin and Eosin. The slides were then examined under a light microscope for evidence of damage at each irradiation site.

The threshold intensity level for lesion production for each animal was taken as the root-mean-square of the highest intensity at which no lesion occurred and the lowest intensity at which a lesion occurred, for a fixed exposure duration, analogous to Dunn *et al.* [8]. Results from several animals were used to define the threshold level for lesion production at each of the several exposure durations.

RESULTS

Intensity levels for focal lesion production in the cat liver have been determined for exposure durations of 0.003, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 20, and 35 s at 3 MHz and are plotted as the circles in Fig. 1. The circles are placed at the means of thresholds obtained for several cats, typically at least 3, and the bar shows the range of those results. The data plotted on Fig. 1 are from animals sacrificed 1 h after irradiation except for the point at 0.03 s exposure duration that is based on data from animals sacrificed at 24 h. For this study the cats were routinely sacrificed at 1 h post irradiation to eliminate the loss of animals, unrelated to the ultrasound, that was common to the 24 h sacrifice studies. Table I shows the comparison between data taken using a 24-h sacrifice time reported previously [6] and data based on the 1-h sacrifice time used almost exclusively in this study. It is readily seen that over the common range of exposure durations, the two sets of data are in good agreement.

A linear regression fit applied to the five data points on Fig. 1 including exposure durations of 0.1 through 10 s yielded the relation $IT^{0.476} = 463 \text{ W/cm}^2 \cdot \text{s}^{0.476}$, where I is the spatial peak delivered intensity and T is the exposure duration. Considering only the data for exposure durations from 0.3 through 3 s yielded the relation $IT^{0.519} = 461 \text{ W/cm}^2 \cdot \text{s}^{0.519}$. In light of the above relations it is apparent that the threshold dosage for lesion production in this range of exposure durations corresponds closely to an $IT^{0.5} = 460 \text{ W/cm}^2 \cdot \text{s}^{0.5}$ relation, represented by the dashed line in Fig. 1. Thus this portion of the curve for

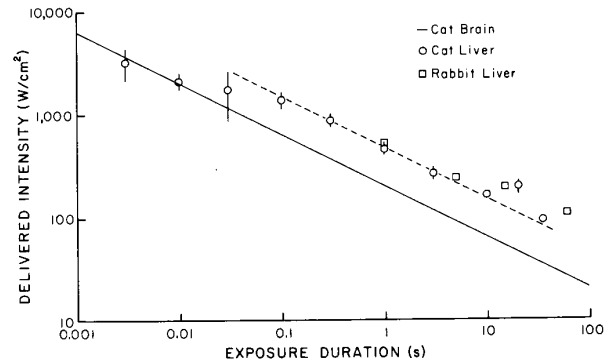


Fig. 1. Threshold data plotted as delivered intensity versus exposure duration for cat brain [2], rabbit liver [5], and cat liver (this study). Cat liver data are at 3 MHz and 37°C.

cat liver has the same slope but occurs at more than twice the intensity as the corresponding curve for the brain that obeys the relation $IT^{0.5} = 200 \text{ W/cm}^2 \cdot \text{s}^{0.5}$ [9], represented by the solid line in Fig. 1. The deviation from an $IT^{0.5} = C$, where C is a constant, dependence of the liver threshold data at shorter and longer exposure durations is considered in the next section of this report. At the longer exposure durations or lower intensities, the results of this study are in excellent agreement with the results reported by Frizzell *et al.* [5] on rabbit liver at 2 and 6 MHz; the 2-MHz data (that closest in frequency) are plotted on Fig. 1.

DISCUSSION

Several points should be emphasized concerning the comparison of the rabbit and cat liver data before discussing the nature of the threshold curves. The excellent agreement between these results which is apparent in Fig. 1, is quite significant for the following reasons: 1) the results were obtained in different species; 2) different frequencies were employed; 3) the rabbits were sacrificed at 3 days post irradiation rather than 1 h; 4) the experimental systems were different, though both studies employed focused fields; and 5) the thresholds for lesion production were defined differently in the rabbit study (50 percent occurrence of a lesion defined the threshold). Thus this comparison suggests species invariance with respect to the liver as has been observed for the brain [2]; provides more support for previously observed frequency independence [10]; and supports the lack of threshold dependence upon time of sacrifice post irradiation. Additional support for the lack of dependence on sacrifice time is provided by the comparison of 1 and 24 h sacrifice data listed in Table I. It also appears that studies employing strongly focused sources will yield the same results even though thresholds obtained with unfocused fields are substantially lower [11]. It appears to matter little whether the threshold is determined as the mean of threshold results from several animals as in this study or as the level for 50 percent probability of lesion occurrence used by Frizzell *et al.* [5].

TABLE I
THRESHOLDS FOR LESION PRODUCTION IN CAT LIVER IN VIVO

Exposure Duration (s)	Threshold Intensity (W/cm ²) at 1 h Sacrifice Time			Threshold Intensity (W/cm ²) at 24 h Sacrifice Time		
	Mean	(Range)	Number of Animals	Mean	(Range)	Number of Animals
0.003	3240	(2090-4360)	3			
0.01	2110	(1900-2420)	3			
0.03	1930	(1480-2690)	3	2130	(1960-2390)	3
0.1	1430	(1110-1600)	3	1400	(1250-1510)	3
0.3				870	(863-876)	2
1	460	(407-516)	3	512	(461-599)	3
3	261	(231-292)	3			
10	160		1			
20	196	(166-210)	4			
35	92		1			

This is probably indicative of a rather sharp threshold as a function of intensity.

The data for exposure durations longer than about 10 s appear to deviate from the $IT^{0.5} = 460 \text{ W/cm}^2 \cdot \text{s}^{0.5}$ dependence observed for shorter exposure durations. This deviation has also been observed for results from the brain at much longer exposure durations [12]. Johnston and Dunn [12] suggested that this deviation might be explained on the basis of a hysteresis effect due to a nonlinear relation between the stress and strain. They applied their hypothesis to the published data on liver, lens of the eye, and brain and showed good agreement between theoretical and experimental results which exhibited a change from 0.5 to a lower power dependence on T for large values of T . However, the differences in local blood perfusion should also be examined to determine if the cooling provided by the blood flow might play a major role in determining the behavior of the curve defining the threshold for lesion production.

At short exposure durations or high intensities, the threshold data for lesion production in the cat liver are lower than would be obtained by extrapolating the fit to the data for exposure durations between 0.1 and 10 s, the dashed line in Fig. 1. In the intensity range between 1000 and 2500 W/cm² the curve for the liver changes such that it appears to follow the curve for the brain at intensities above 2500 W/cm². This transition from one level to another is highly suggestive of a change in the dominant mechanism for production of the damage in the two regions, i.e., above 2500 W/cm² versus below 1000 W/cm². This transition range coincides with the range, when increasing intensity, where the histological evidence suggests a transition to a cavitation mechanism as the dominant mechanism of damage. Above this intensity range the lesions in both the brain [4] and the liver [6] are quite different in their appearance. In the cavitation range the lesions in both organs have irregular boundaries, exhibit much more destructive damage to the point of complete homogenization of the destroyed tissue, and may occur preferentially at boundaries. Conversely, below the

cavitation range the lesions have smooth boundaries, exhibit less mechanical damage to the tissue, and always occur at the center of the focal region. Thus the new data for cat liver present additional and, when considered in conjunction with the results noted above, quite conclusive evidence for the occurrence of a change in the dominant mechanism of damage in the intensity range 1000-2500 W/cm². Additionally, these data indicate that the threshold for focal lesion production by a cavitation process in the liver and the brain may be identical. Thus whatever constitutes the nuclei for the cavitation events may be common to these and perhaps most parenchymal tissues.

Based on histological evidence of preferential occurrence of cavitation lesions at boundaries one might speculate that the nuclei are associated with the body fluids. Some credence is lent to this hypothesis by the threshold results in the lens of the eye [13] where the mechanism of damage appears to be thermal to the highest intensities used, 1500 W/cm². The character of the lens is quite different from the brain and liver in that it has no vascular system and is a polycrystalline solid. It is possible that cavitation lesions have not been observed because a vascular system containing cavitation nuclei is not present. It would be interesting to observe lesions at higher intensities in the lens to verify that cavitation lesions do not occur at slightly higher intensities.

Finally it should be noted that it is likely that nonlinear phenomena, specifically harmonic generation and associated increased absorption, are involved at the highest intensities used in this study. Most of the sound path was low absorbing Ringer's solution which would allow the generation of harmonics as observed by Goss and Fry [14] for focused sound fields in water. However, it is not evident that any differences in nonlinear effects led to significant differences in lesion thresholds between liver and brain (where the portion of the sound path in Ringer's solution was usually less than for liver). Also, similar thresholds were obtained using the brain of a rat [3], [15] and cat [1], [2] where the relative water and tissue paths can differ significantly.

SUMMARY

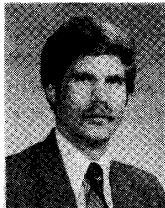
The threshold for high intensity focal lesion production has been determined at 3 MHz and for exposure durations from 0.003–35 s in the cat liver. The liver threshold curve was found to parallel that for the brain over the approximate exposure duration range 0.1–10 s but to be more than twice the intensity level, being approximately described by the relation $IT^{0.5} = 460 \text{ W/cm}^2 \cdot \text{s}^{0.5}$. For longer exposure durations the liver threshold curve exhibits a lesser power dependence on the exposure duration. The fact that this occurs at shorter exposure durations than for the brain may be explained on the basis of a hysteresis effect due to a nonlinear relation between stress and strain [12], but the effect of any differences in local blood perfusion should be investigated. At exposure durations below 0.1 s, at intensity levels above 1000 W/cm^2 , the liver threshold curve goes through a transition region intersecting the brain threshold curve between 2000 and 2500 W/cm^2 and then following the brain threshold. This behavior at high intensities provides quite conclusive evidence for a change in the primary mechanism of damage and tends to confirm that a transient cavitation mechanism of damage becomes dominant at intensities above 2500 W/cm^2 . It also suggests that the cavitation nuclei may be similar for the two tissues.

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