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The Disruption of Murine Tumor Neovasculature by Low-intensity Ultrasound – comparison between 1 MHz and 3 MHz sonication frequencies ¹

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Abstract

Objective—The goal was to determine whether the tumor vascular disrupting actions of low-intensity ultrasound were frequency dependent.

Materials and Methods—The effect of the frequency (1 MHz at 2.2 W.cm⁻² or 3 MHz at 2.4 W.cm⁻²) of low intensity ultrasound as a neovascular disrupting modality was investigated in 15 murine melanomas (K1735²²) insonated for three minutes following the intravenous injection of a microbubble contrast agent (Definity). In contrast-enhanced power Doppler observations of each tumor (before and after treatment) measurements were made of the size of the area of the tumor that was perfused with blood containing the ultrasound contrast agent (percentage area of flow [PAF]), and the volume of contrast agent flowing through the unit volume of the tumor (color weighted fractional area [CWFA]). During insonation of the tumor the temperature was measured with a fine wire thermocouple in an additional eight mice.

Results—The antivascular action of low-intensity ultrasound was significantly enhanced (PAF by 64%; CWFA by 106%) when the tumor was treated with 3 MHz ultrasound rather than 1 MHz (ANOVA: PAF - $P = 0.02$; CWFA - $P = 0.04$). The average rate of tumor temperature increase was 2.6 ± 1.3 °C.min⁻¹ for 1 MHz and 5.0 ± 1.7 °C.min⁻¹ for 3 MHz; these increases were significantly different ($P = 0.04$).

Conclusions—Insonation of the tumor at a higher frequency amplified the heating of the neoplasm and led to greater disruption of the tumor vasculature; 3 MHz ultrasound was more efficacious than 1 MHz for antivascular cancer therapy.

Keywords

Ultrasound antivascular effects; vascular disrupting agent; ultrasound microbubble contrast agent; low-intensity ultrasound therapy; cancer therapy

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INTRODUCTION

The influence of low-intensity, physiotherapy ultrasound beams on the growth of neoplasms has been investigated. In a rhabdomyosarcoma in mice it was found that the neoplasms receiving 10 five minute treatments over two weeks with a continuous 3 MHz beam at 1 W.cm^{-2} grew at a faster rate than the control, untreated tumors (1). Such an increase in growth may have been related to an enhanced angiogenic response similar to that observed following the insonation (15 or 30 min, 1.0 MHz, 20% duty cycle, 2.2 W.cm^{-2}) of endothelial cells in tissue culture (2). Subsequent rhabdomyosarcoma studies showed that 3 MHz beams of 0.75 W.cm^{-2} (continuous) and 1.5 W.cm^{-2} (pulsed) had no effect on tumor growth and at necropsy there were no histologic changes related to the therapy (3).

As opposed to sonication alone, combination of microbubble-containing contrast agent, and low-intensity ultrasound acted as a potent tumor vascular disrupting agent (4–6). Treatment of a murine melanoma with a low-intensity beam (1MHz, continuous, $I_{\text{SATA}} = 2.3 \text{ W.cm}^{-2}$) reduced tumor blood flow¹ by 25% for each minute of insonation (4). Histology showed that insonation lead to an acute apparently irreparable dilation of the tumor capillaries with associated hemorrhage and intercellular fluid (5). These capillaries had formed by angiogenesis (7–10) - following the up-regulation of the *angiogenic switch*, new vessels sprouted from the adjacent normal vasculature to supply the tumor. In the presence of the ultrasound contrast agent, the tumor capillaries appeared uniquely sensitive to low-intensity ultrasound (6). Twenty four hours after insonation, liquefactive necrosis of the neoplastic cells (related to tumor ischemia) occurred. Also, histology demonstrated that pre-existing structures, including arterioles and venules, that had been enveloped by the neoplasm were unaffected by the treatment (5). It seemed that there was an interaction between the low-intensity ultrasound beam, the microbubble contrast agent and the tumor neovasculature (6). Several previous studies (11 and the references therein) have demonstrated that tissue heating is enhanced when contrast microbubbles are present at the time of sonication. It would be expected that an increase in ultrasound frequency would increase thermal bioeffects (12–14), either by increasing the absorption of ultrasound energy directly and/or by its absorption via circulating microbubbles (15,16). On the other hand, following an increase in sonication frequency there would be a reduction in the incidence and importance of the non-thermal bioeffects associated with inertial cavitation (in which microbubbles of the contrast agent expand and then collapse in the presence of insonation) (17,18).

It is currently not known how the ultrasound induced antivasular activity, which we have described in our earlier studies (4–6) using 1MHz, is affected by sonication frequency. The goal of this study was to further characterize the function of low-intensity ultrasound as a tumor vascular disrupting agent and to determine whether its action is affected when the sonication frequency is changed to 3MHz.

MATERIALS AND METHODS

Tumor model and ultrasonography

As described previously (4–6), two million murine melanoma cells (K1735²²) were injected subcutaneously in the right flank of 15 female mice (C3HV/HeN strain; Charles River Laboratories, Wilmington, MA, USA). The tumor model was approved by the University's Institutional Animal Care and Use Committee. For the ultrasonographic studies a tail vein was

¹Contrast-enhanced Doppler imaging used in this study assesses blood flow by measuring the fractional area of tumor enhanced by the contrast agent and the magnitude of enhancement. These properties of Doppler images are intimately related to fractional blood volume or tumor perfusion and in this article we have used these terms interchangeably.

catheterized and general anesthesia was induced and maintained with isoflurane (IsoSol, VEDCO Inc, St Joseph, MO, USA). B-mode ultrasonographic and contrast-enhanced power Doppler observations were made (7–15 MHz probe; HDI 5000 SonoCT, Philips, Bothell, WA, USA) of each tumor once it had grown to at least 9 mm in one dimension over 22 to 43 days.

Tumor insonation and experimental design

Each tumor was insonated with a physiotherapy ultrasound machine (1 or 3 MHz, continuous output, power level = 2; D150 Plus, Dynatronics Corp., Salt Lake City, UT, USA) for three min. The effective intensity of the ultrasound beam was measured using a radiation force balance (UPM 30, Ultrasound Power Meter, Ohmic Instruments Co, St Michaels, MD, USA; [4]). At 1MHz the intensity of the beam (I_{SATA}) was 2.2 ± 0.1 ($n = 3$) W.cm^{-2} and at 3 MHz it was 2.4 ± 0.1 ($n = 3$) W.cm^{-2} . Sonographic gel was applied to the skin overlying the tumor and the insonation was made by direct contact between the transducer and the tumor; the transducer had an effective radiating area of 7 cm^2 . There was a 1 minute gap between each minute of insonation, during which time the face of the physiotherapy transducer was placed in water at room temperature.

The mice were randomly divided into three groups as follows:

1. In the control group (group 1; $n = 5$) a sham therapy was performed. The initial B-mode study (in which the size of the tumor was measured) was followed by the intravenous injection of 0.01 mL of an ultrasound contrast agent (Definity, Bristol-Myers Squibb Medical Imaging, North Billerica, MA, USA) and minimum exposure contrast-enhanced power Doppler (minexCPD) was performed to assess tumor blood flow. minexCPD was performed by gating the ultrasound scanner (ATL Philips 5000, Bothell, WA, USA) externally at $0.5 \text{ frames.s}^{-1}$. Low frame image acquisition reduced ultrasound exposure and minimized bubble destruction (4,19). To ensure high quality images, minexCPD was performed at an MI of 0.8 using the highest dynamic range available on the scanner. The use of low dose of contrast agent combined with the highest dynamic range helped in minimizing the potential blooming artifacts. After the contrast agent was no longer detectable in these initial power Doppler observations, a further 0.2 mL Definity was injected and the physiotherapy transducer was applied to the tumor (for three one minute periods) but was not switched on. The mouse then recovered from the general anesthetic. A day later the B-mode and contrast enhanced power Doppler studies were repeated.
2. In the first treated group (group 2; $n = 5$), the initial B-mode and contrast-enhanced power Doppler studies were followed by tumor insonation with a low-intensity beam of 1 MHz. As in the control group, the B-mode and contrast enhanced power Doppler observations were repeated 24 hours later.
3. In the second treated group (group 3; $n = 5$), a similar study to group 2 was performed, however, the frequency of the ultrasound beam used for insonating the tumor was 3 MHz. The enhancement of all power Doppler images was digitally recorded for quantitative analysis.

Analysis of contrast-enhanced power Doppler images

The size of the area perfused in each neoplasm was measured from the contrast-enhanced power Doppler ultrasonographic images prior to and 24 hours following insonation. The following methods were used:

- i. percentage area of flow (PAF)

A software program identified the number of colored pixels (n) within the power Doppler image of the tumor that demonstrated maximum contrast-enhancement (20, 21). The PAF or percent area covered by colored pixels was calculated as $n.100/N$, where N is the total number of pixels in the contrast enhanced power Doppler image. The PAF represented the size of the area of the tumor that was perfused with blood containing the ultrasound contrast agent.

ii. color weighted fractional area (CWFA)

The CWFA was obtained from the maximally enhanced power Doppler images by weighting each colored pixel in the PAF by its color level (20,21). If there are n_i pixels of color levels ' i ', the CWFA was determined by the formula:

$$CWFA = \frac{\sum_{i=1}^{i=100} in_i}{N}$$

Because the color level of pixels in the power Doppler image is related to the concentration of moving scatterers, CWFA is a measure of the volume of contrast agent flowing through the unit volume (voxels) of the tumor within the image plane.

A paired t -test (MedCalc Software, Mariakerke, Belgium) was used to determine within groups whether there was any significant effect of insonation on tumor vascularity as assessed by a PAF and CWFA. The parameters for each group of mice were recorded as a mean $\pm$ SD. An ANOVA (StatView, SAS Institute Inc., Cary, NC, USA) was used to analyze the differences between the mean change in vascularity observed between the different groups of mice. In each analysis the difference was considered significant when $P \leq 0.05$.

***In vivo* estimations of tumor heating**

In other mice the temperature of the tumor was measured during insonation. Prior to imaging and therapy, a fine wire thermocouple (0.08 mm diameter; Omega Engineering Inc, Stamford, CT) was inserted into the center of the tumor using an 18 gauge needle. The needle was withdrawn and the central position of the thermocouple was confirmed in B-mode images. In groups of four mice, an identical imaging and therapy protocol was followed to that described for Group 2 (1 MHz therapy) and 3 (3 MHz therapy) tumors. The temperature (T_i) in degrees Celsius of the tumor was measured every two seconds during each minute of insonation and displayed on a computer monitor using data recording software (Omega Engineering Inc, Stamford, CT). The mean temperature increase ($\Delta\bar{T}$) for each frequency of sonication was determined from the temperature–time (t) profile using the following formula:

$$\Delta\bar{T} = \frac{\int_0^t \Delta T_i dt}{\int_0^t dt},$$

where ΔT_i is the increase in temperature due to heating. The peak increase in temperature during each insonation was also recorded.

In addition thermal dose, was measured by the following formula (22,23).

$$TD = \int_0^t R^{(T-43)} dt,$$

Where TD = thermal dose, $R=2$ for $T \geq 43^{\circ}\text{C}$ and $R=4$ for $37^{\circ}\text{C} < T < 43^{\circ}\text{C}$. The mean $\pm$ SD of each of the temperature and TD measurements for each frequency were determined and the statistical significance of the difference between frequencies was established by an unpaired *t*-test assuming unequal variances (MedCalc Software, Mariakerke, Belgium). Each mouse was euthanized at the completion of the experiment.

Histology

Following therapy and imaging, each of the 15 mice was euthanized, and the tumor was removed, fixed, and embedded in paraffin; the tumor from one of the sham-treated mice was not available for histology. The mid-portion of each neoplasm corresponding to the imaging region was sampled, stained with hematoxylin and eosin, and viewed with a light microscope at standard clinical diagnostic magnifications. The histopathologist reviewing the tissue samples was blinded to the treatment given to each neoplasm. In each histologic slide, the absence or presence of dilated tumor capillaries, hemorrhage, dissociation of the neoplastic cells, necrosis of neoplastic cells, and pseudocyst formation was determined by visual inspection. The % area of the total surface of the neoplastic tissue affected by each histological change in the slide was recorded. From these measurements, the average total % area affected in the histologic specimens of each neoplasm and the mean $\pm$ SD of the measurements for each group of mice were calculated (5). The extent of increased intercellular fluid was also quantified and expressed as a percentage area affected and a score (absent: score = 0; mild: score = 1; moderate: score = 2; severe: score = 3); the percentage area and scores in the histologic slides of each mouse were averaged and the means $\pm$ SD for each group of mice were calculated. Data from different treatment groups were compared by ANOVA (StatView, SAS Institute Inc., Cary, NC, USA) to determine whether the total % area affected by histologic change, the incidence of the individual histologic findings, and the amount of intercellular fluid were statistically significant. The differences were considered significant at $P \leq 0.05$. A linear regression analysis (StatView, SAS Institute Inc., Cary, NC, USA) was performed to evaluate whether there was a correlation between the total % area of histological change and the % decrease in tumor vascularity following insonation (as demonstrated by PAF and CWFA). The presence of pre-existing structures (arterioles, venules, nerves, adipocytes, skeletal muscle fibers and mammary ducts) within the neoplasm was also recorded.

RESULTS

Clinical and Doppler findings

Each of the mice recovered normally from the general anesthetic used for the initial imaging and therapy. All but one were also normal the following day – a single mouse, whose tumor was treated with 3 MHz, was depressed and there were three 1–2 mm irregularly-shaped cutaneous hemorrhages overlying the tumor. Following the sham-treatment in the control mice, there was no change in tumor vascularity (Table 1, Fig. 1). However within the treated groups, there were significant reductions in the blood flow in the tumors insonated at either 1 or 3 MHz (Table 1, Fig. 1). The ANOVA of the changes in tumor vascularity between treatment groups showed significant reductions in tumor blood flow between the sham-treated tumors and each of the treated groups (sham treated vs 1 MHz: PAF - $P = 0.006$; CWFA - $P = 0.03$; sham treated vs 3 MHz: PAF - $P = 0.0001$; CWFA - $P = 0.0003$; Fig 2). An enhanced role of a 3 MHz beam as a vascular disrupting agent when compared to a 1 MHz beam was also demonstrated; PAF decreased by 64% and CWFA by 106% (1 MHz vs 3 MHz: PAF - $P = 0.02$; CWFA - $P = 0.04$; Fig 2).

In vivo estimations of tumor heating

In the *in vivo* studies there was no change in temperature of the tumor during either the pre- or post-treatment B-mode or contrast enhanced power Doppler observations. There were however

significant increases in tumor temperature during treatment. The mean temperature rise for each minute of insonation was 2.6 ± 1.3 °C.min⁻¹ for the 1 MHz beam and 5.0 ± 1.7 °C.min⁻¹ for 3 MHz; these increases were significantly different ($P = 0.04$). The peak temperature rise in the tumors was 5.2 ± 1.4 °C for the 1 MHz beam and 10.2 ± 2.7 °C for 3 MHz; these increases were significantly different ($P = 0.02$). Considerable variations between tumors were observed in measurement of thermal dose (17 ± 28 min [median= 8.5min] for 1 MHz sonication and 664 ± 1129 min [median 304 min] for 3 MHz sonication); these changes were not statistically significant.

Histologic findings

The untreated neoplasms showed distinct, unencapsulated margins and were characterized by closely spaced melanoma cells (Fig 3). Following therapy there were significant increases in the total % area of histologic change both between the control and treated groups and also between tumors treated with 1 and 3 MHz ultrasound (Table 2). The predominant histologic findings were dilation of the tumor capillaries (often with associated mild intercellular edema), followed by hemorrhage, and dissociation of the neoplastic cells (Figs 4 and 5); necrosis of the neoplastic cells was less common and the presence of pseudocysts was considered to be characteristic of the neoplasm and not related to the treatment (Table 2). In the regions of cellular disociation the melanoma cells were separated by abundant edema and were rounded in shape; the cells appeared either uninjured, degenerated or necrotic (Fig 5). Note that at this acute stage the dissociated neoplastic cells were not all necrotic as many nuclei were neither pyknotic, karyorrhectic nor karyolytic, and the cytoplasm did not demonstrate increased eosinophilia; all of these histologic changes characterize tumor cell necrosis. The linear regression analyses demonstrated significant correlations between the total percentage area affected by histologic change and the contrast enhanced power Doppler estimations of tumor blood flow (histology vs PAF: correlation coefficient = -0.74 , $P = 0.003$; histology vs CWFA: correlation coefficient = -0.67 , $P = 0.009$).

Across all tumors the presence of intercellular fluid was variable, and no well-defined patterns of incidence and severity could be defined; such a change was interpreted to be unrelated to insonation but was a feature of the growth of the neoplasm (Table 2). Pre-existing structures (arterioles, venules, nerves, adipocytes, skeletal muscle fibers and mammary ducts) that had been enveloped by the growing neoplasm were variably detected across each group; each was unaffected by the insonation.

DISCUSSION

This study confirmed our earlier findings that low-intensity ultrasound disrupts tumor vasculature in the presence of a microbubble contrast agent (4–6). The antivascular action was significantly enhanced when the tumor was treated with 3 MHz ultrasound rather than 1 MHz. There were significant correlations between the estimations of tumor blood flow, made from the contrast enhanced power Doppler studies, and the extent of histologic change; that is, the histological changes were more extensive in tumors treated with 3 MHz ultrasound than with 1 MHz. The predominant acute effects of insonation at 1 and 3 MHz were dilation of the tumor capillaries and hemorrhage. Vessel dilation has also been observed during hyperthermia (24–28) and treatment of tumors with doxorubicin (29). The mechanism of capillary dilation is not completely understood, however, it is possible that the treatment injured the endothelial cells and pericytes. These cells, which have a weak, fragile, incomplete arrangement in the neovasculature of the tumors, could be damaged and so could no longer provide the normal structural (or mechanical) support for the capillary. As the vessel wall is no longer functional it could be incapable of counteracting the intravascular pressure, and thus the capillary dilates and becomes leaky.

In this study, dissociation of the neoplastic cells (in which the tumor cells were widely separated by edema and became rounded in shape) was an acute change. It had not been described in our previous studies (4–6) and was thought to be related to an abrupt, irreversible cellular injury that would eventually lead to neoplastic cell death. Because this unusual histologic finding of a mixture of uninjured, degenerated and necrotic cells in a pool of edema was an immediate effect of tumor insonation, it was likely due to a direct bioeffect of ultrasound, rather than secondary to ischemia that occurs over time following the vascular changes.

A common interaction between tissues and ultrasound occurs by the absorption of acoustic energy as heat (14,30). The ultrasound absorption by tissues increases almost linearly with frequency (31,32). In the absence of an ultrasound contrast agent, *in vitro* observations have shown that 3 MHz ultrasound caused a significantly greater increase in temperature than 1 MHz ultrasound (12–14). The rates of temperature increase in *in vivo* studies of human muscle have ranged from $0.38^{\circ}\text{C}\cdot\text{min}^{-1}$ (1.0 MHz and $2.0\text{ W}\cdot\text{cm}^{-2}$; [33]) to $1.4^{\circ}\text{C}\cdot\text{min}^{-1}$ of insonation (3.0 MHz and $2.0\text{ W}\cdot\text{cm}^{-2}$; [33]); in this study, the mean temperature rise was $2.6^{\circ}\text{C}\cdot\text{min}^{-1}$ for 1 MHz and $5.0^{\circ}\text{C}\cdot\text{min}^{-1}$ for 3 MHz. The observation that 3 MHz sonication caused greater neovascular disruption than 1 MHz and also lead to greater temperature and thermal dose suggests that the ultrasound-induced antivascular activity is correlated to direct heating of the tumor due to ultrasound absorption.

Additionally, interactions through inertial cavitation are also possible but require insonation at high intensities (11,34–37). However, the presence of microbubbles in a medium can induce inertial cavitation at lower sonication intensities. In liquids, inertial cavitation has been observed at a pressure amplitude of 0.58 MPa in the presence of a contrast agent (38). In rabbit ear vessels, inertial cavitation activity has been reported at a pressure amplitude of 1.1 MPa using pulsed ultrasound in the presence of microbubble contrast agents (39). Whether inertial cavitation occurred at the intensity used in our tumor study ($\sim 2.4\text{ W}\cdot\text{cm}^{-2}$, pressure amplitude 0.27 MPa) has not yet been demonstrated. If inertial cavitation were to occur, it could also contribute to the observed antivascular activity. Several previous reports, however, have demonstrated that inertial cavitation is diminished when sonication frequency is increased (13). The threshold intensity for inertial cavitation increases with frequency. Thus if inertial cavitation alone was responsible for the antivascular effect, one would anticipate a reduced incidence of vascular disruption at 3 MHz. The observation that there was greater antivascular action at 3 MHz than at 1 MHz suggests that inertial cavitation may not have contributed significantly to the vascular disruption observed in our study. Further, in extrapolating the results of other *in vivo* observations (39), in which microbubbles were circulating through rabbit auricular vessels, the estimated half-life of inertial cavitation was likely to be only a few seconds in duration at 0.2 MPa (a pressure amplitude similar to that of the 1 and 3 MHz ultrasound used in this study). Since the insonation time used in this study was 180s, it once again suggests that inertial cavitation may not have been a dominant factor in the observed antivascular effects.

In conclusion, the results of this study showed that the ultrasound antivascular activity increased significantly when the sonication frequency was changed from 1 MHz to 3 MHz. Following the injection of an ultrasound microbubble contrast agent, insonation of a murine tumor with low-intensity ultrasound at a higher frequency amplified the heating of the neoplasm and led to greater disruption of the tumor vasculature. The histologic changes were also more extensive at the higher frequency. All three findings (tissue heating, tumor vascular changes and tumor histology) suggest that 3 MHz ultrasound was more efficacious than 1 MHz for antivascular therapy.

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Figure 1.

Power Doppler ultrasound images of murine melanomas following the intravenous injection of an ultrasound contrast agent, before ([a], [c], [e]) and 24 hours after ([b], [d], [f]) treatment with low intensity ultrasound. Each image shows the region from which the Doppler information was acquired, and the boundaries of the neoplasm, traced in white from the corresponding B-mode image, have been copied to these images. The distance between the arrow heads in the left column represents a 5mm scale. In the control tumor there is no significant change in tumor vascularity following the sham treatment (compare images [a] and [b]; group 1, mouse H1). Significant reductions in tumor blood flow were, however, observed following treatment with low intensity ultrasound at 1 MHz (compare images [c] and [d]; group 2, mouse F1) and 3 MHz (compare images [e] and [f]; group 3, mouse E4).

Figure 2.

Changes in murine tumor vascularity in contrast enhanced power Doppler ultrasound images after treatment with low intensity ultrasound. Following therapy, the light gray blocks represent the mean size of the change in vascularity as measured by percentage area of flow (PAF), and the dark gray blocks as measured by color weighted fractional area (CWFA). There were no significant changes in PAF or CWFA following the sham treatment. The ANOVA, however, showed significant reductions in tumor blood flow (overall PAF – $P = 0.002$, overall CWFA – $P = 0.001$). Following therapy with the 1 and 3 MHz low intensity ultrasound beams (in comparison to the sham treated group) were significant (1 MHz: PAF – $P = 0.006$, CWFA – $P = 0.02$; 3 MHz: PAF – $P = 0.001$, CWFA – $P = 0.003$). The increased antivascular effect of the 3 MHz treatment in comparison to the 1 MHz treatment was also statistically significant (PAF – $P = 0.02$, CWFA – $P = 0.04$).

Figure 3.

The untreated melanoma (group 1, mouse D3) demonstrates a typical histologic pattern characterized by closely spaced neoplastic cells forming a mass with a sharply demarcated unencapsulated border subjacent to the subcuticular tissue (a; three arrows). At a higher magnification (b) the closely spaced short spindle cells form interconnecting bundles or streams with unapparent vasculature.

Figure 4.

In a tumor insonated at 1 MHz (a; group 2, mouse F1), there is marked locally-extensive capillary dilation (the three solid arrows show three closely spaced capillaries) and hemorrhage (examples outlined by two ovals), and there is also a large focal area (three open arrows) demonstrating dissociation of the melanoma cells by edema with accompanying similar

capillary dilation and hemorrhage. The higher magnification (b) shows severe capillary dilation (four solid arrows) and hemorrhage (circle) with mild intercellular edema (two open arrows).

Figure 5.

In a tumor insonated at 3 MHz (a; group 3, mouse D4), there is an interface (three arrows) between an upper region showing mild hemorrhage and a lower region demonstrating severe disassociation of the melanoma cells by edema with moderate capillary dilation and hemorrhage.

At a higher magnification (b) of the region of dissociation, the individual melanoma cells are widely separated by abundant edema and are mostly rounded in shape. Some neoplastic cells have degenerated as shown by their vacuolated cytoplasm (three solid arrows), some are necrotic as demonstrated by their pyknotic nuclei and eosinophilic cytoplasm (three open arrows), and the remainder appear uninjured. An area of hemorrhage is also present (two arrow heads).

Table 1
Effect of sonication on murine tumor vascularity (measurements within groups from contrast-enhanced power Doppler images)

Group	Sonication frequency	PAF(%)			CWFA(%)		
		Pre-treatment	Post-treatment	P	Pre-treatment	Post-treatment	P
1	Sham	61.9 ± 7.2	65.3 ± 14.9	0.61	26.1 ± 7.8	31.3 ± 10.4	0.23
2	1 MHz	64.7 ± 21.1	28.8 ± 26.5	0.016	32.6 ± 14.4	14.1 ± 15.4	0.009
3	3 MHz	69.7 ± 24.0	14.6 ± 12.2	0.017	39.7 ± 22.0	6.4 ± 6.3	0.027

Key: PAF = percentage area of flow; CWFA = color weighted fractional area. Data analyzed by paired t-test.

Table 2

Summary of histologic changes in murine melanomas

Group	Dilation % area	Hemorrhage % area	Disassociation % area	Necrosis % area	Cyst % area	Intercellular fluid* % area	Score
1	0.2 ± 0.3	1.5 ± 1.2	1.6 ± 1.7	1.0 ± 1.1	0.3 ± 0.5	12.2 ± 8.5	1.1 ± 0.6
2	33.2 ± 23.0	27.2 ± 20.1	25.4 ± 22.9	6.8 ± 4.5	0.1 ± 0.2	19.6 ± 10.7	1.7 ± 1.0
3	69.0 ± 11.5	45.3 ± 11.3	41.3 ± 22.2	10.2 ± 3.0	1.2 ± 2.7	5.4 ± 4.1	1.0 ± 0.8
ANOVA P-value	0.0001	0.002	0.03	0.006	0.54		
1,2 P-value	0.009	0.02	0.09	0.02	0.89		
1,3 P-value	<0.0001	0.0007	0.01	0.002	0.41		
2,3 P-value	0.004	0.06	0.22	0.14	0.31		

* no well-defined patterns of incidence and severity were observed.