# Sonodynamic therapy: an approach to tumor treatment utilizing sonochemical activation

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## ABSTRACT

Sonodynamic therapy was proposed on the finding that certain chemicals such as hematoporphyrin are activated by acoustic cavitation and thereby induce a significant antitumor effect. It was also found that sonodynamically active cavitation can be induced at a relatively low ultrasonic intensity through superimposing the second harmonic onto the fundamental. The intensity threshold for producing focal damage in murine liver was reduced by orders of magnitude through second-harmonic superimposition especially in combination with administration of a certain xanthene. The effect was maximized at a second-harmonic phase emphasizing the peak rarefaction, which is consistent with the hypothesis that microbubble growth rather than collapse was accelerated.

## INTRODUCTION

Ultrasonically induced cavitation is known to be the primary mechanism of the nonthermal bioeffects of ultrasound. Such bioeffects must be avoided in ultrasonic diagnosis, but may have potential use in therapeutic application of ultrasound. Acoustic cavitation is also known to be the mechanism of sonochemical effects. *In vitro* and *in vivo* experiments demonstrated that ultrasound can activate certain porphyrins and thereby induce significant antitumor effects [1-4] However, these experiments were performed under standing wave conditions. Standing waves can induce acoustic cavitation much more easily than by progressive waves, but progressive waves can be focused on a tumor at locations of much more variety than standing waves.

Recently, we found that acoustic cavitation can be efficiently induced, even by progressive waves, if the second harmonic is superimposed onto the fundamental [5-8]. Second-harmonic superimposition decreased the intensity threshold for producing in vivo tissue damage paired with fractional harmonic emission by more than an order of magnitude, especially with administration of a certain xanthene dye [9]. A hypothesis for the mechanism of enhancing cavitation was suggested that the growth of microbubbles under acoustic pressure can be emphasized by a certain asymmetric waveform synthesized by superimposing the second harmonic at a proper phase relative to that of the

fundamental [6].

## MATERIALS AND METHODS

### Sonodynamically active agents

The sodium salt of erythrosin were purchased from Wako Junyaku Kogyo (Tokyo, Japan). Aqueous solution of a gallium-porphyrin complex, 7,12-bis(1-decyloxyethyl)-Ga(III)-3,8,13,17-tetramethyl-porphyrin-2,18-dipropionyl diaspartic acid (ATX-70) dissolved in phosphate buffer (pH 7.3), was supplied by Toyo Hakka Kogyo (Okayama, Japan). Erythrosin was dissolved in phosphate-buffered saline (pH 7.3). Each aqueous solution of sonodynamically active agent was administered to the mice through the tail vein. Erythrosin was administered 15 min before insonation at a dose of 50 mg/kg and 5 mg/kg. ATX-70 was administered 24 h before insonation at a dose of 5 mg/kg.

### Dual-frequency focused array transducer [8]

Focal ultrasonic field with second-harmonic superimposition was produced using a dual-track focused transducer. The fundamental and second harmonic were generated on its co-focally aligned outer and inner tracks, respectively. Here, 0.5 MHz was chosen for the fundamental frequency. A wideband focused hydrophone (Toray Techno, Kanagawa, Japan) was also co-focally located in the central hole of the transducer so as to detect fractional harmonic emissions from the focal zone. The air-backed PZT elements (C-213, Fuji Ceramics, Shizuoka, Japan) of the 2-track, 16-sector array transducer were tightly bonded with a conductive epoxy onto the machined flats formed on the back surface of a spherical aluminum shell. The shell was 100 mm in diameter and had a spherical curvature of 108 mm for geometric focusing. Each transducer element was driven by a high-voltage amplifier consisting of a complementary pair of power MOSFETs. The phase of the input signal to each amplifier was computer-controlled so that the phase of the second harmonic relative to the fundamental was adjusted and that the focal spots at both frequencies were laterally shifted. The supply voltage to the power MOSFET amplifiers was separately adjustable for the outer and inner tracks so as to control the fundamental and second-harmonic acoustic amplitudes independently. The acoustic pressure distribution on the focal plane, produced with each track of the transducer, was measured in degassed water with a needle-type hydrophone at a low acoustic pressure level. The focal spots were 5.6 mm and 4.8 mm in diameter at 0.5 MHz and 1 MHz, respectively. The peak intensity divided by each focal-spot average intensity was 3.84 at 0.5 MHz and 4.58 at 1 MHz. The second-harmonic phase relative to the fundamental was calibrated by measuring the waveforms which were produced with both inner and outer tracks driven at the same time. The absolute acoustic power from each transducer track was calibrated by measuring the radiation force.

### Insonation setup

The mouse was held vertically in degassed saline in the tank as shown in Fig. 1. The saline level in the insonation tank was kept below the mouth to allow easy respiration. Angles between the transducer axis, the tank walls, and the saline surface were chosen for minimal formation of standing waves. The saline was degassed not only right before being put in the tank but also continuously during the experiment by a degasser unit (ERC-3302W, Erma Cr., Tokyo, Japan) in order to prevent acoustic cavitation in saline itself during the insonation. The dissolved oxygen content in saline in the tank was thereby kept below 2 ppm, which was verified by measuring it with an oxygen electrode. Acoustic emission from the insonated tissue was simultaneously detected by the focused hydrophone during every insonation and the signal was processed by a spectrum analyzer (3588A). The temperature rise in the tissue during insonation was checked with a 0.25-mm diameter sheathed chromel-almel thermocouple (Sukegawa Electric, Ibaraki, Japan) inserted into the tissue.

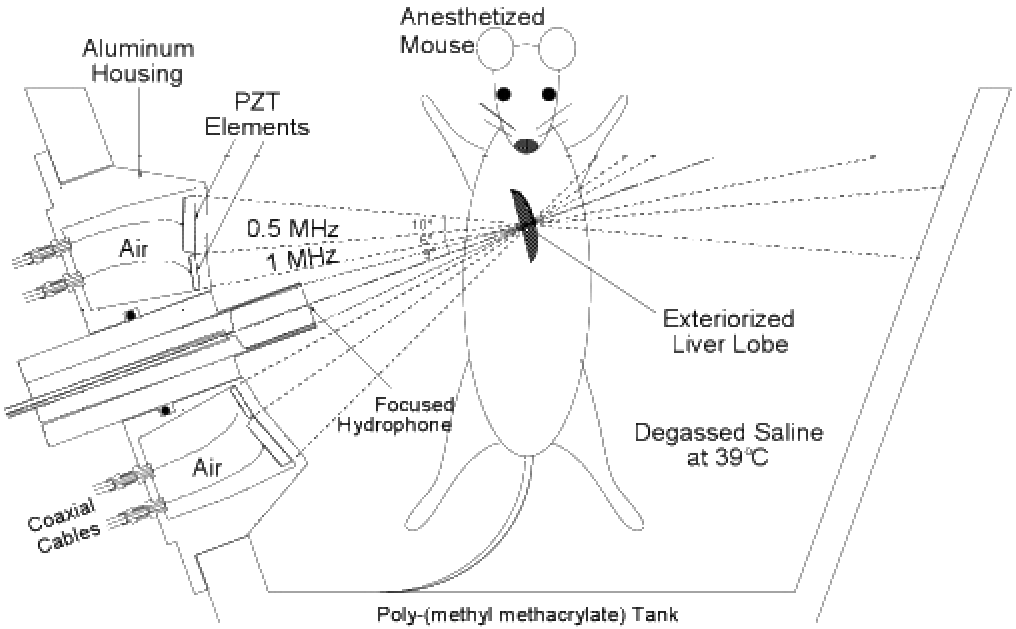


Fig. 1 Dual-frequency focused array transducer attached to water tank for *in vivo* experiment of insonation with second-harmonic superimposition. Acoustic emission from the focal zone is detected with the coaxially located hydrophone. An exteriorized mouse liver lobe or a subcutaneously implanted murine tumor was located at the focus in degassed saline in such a way that the focused ultrasound propagated through it.

Insonation of murine liver [8]

After surgical anesthesia with sodium pentobarbital was given to a five-week-old male ddY mouse (25-30 g), a liver lobe was mobilized and exteriorized through an upper midline incision. The exteriorized liver suspended with a thin suture so that the liver lobe was sticking out through the slit perpendicularly to the abdominal wall. The position of the mouse was adjusted to locate the lobe at the focal spot, and its angle was adjusted to align the lobe parallel to the focal plane. This let the focused ultrasound propagate perpendicularly through the lobe, 23 mm in thickness, without any other acoustic interference. Three mice were insonated at the same condition, and each insonation was continued for a maximum of 3 min until tissue damage was observed.

Insonation of murine tumor

Lewis lung carcinoma was supplied by Research Institute for Tuberculosis and Cancer, Tohoku University. Each transplanted tumor was initiated by subcutaneous trocar-injection of an approximately 1-$mm^3$ piece of fresh tumor into the left dorsal scapula region of a 5-week-old male BDF1 mouse weighing 25-30 g under light ether anesthesia. A week later, when the tumor diameters reached approximately 1 cm, the tumors were treated. A Ga-porphyrin, ATX-70, was intravenously administered 24 h before insonation. Under anesthesia with pentobarbital, a tumor bearing mouse was placed in degassed water at 37°C with its subcutaneously transplanted tumor suspended in the focal zone so that the focused ultrasound propagate through the tumor and the skin without any other acoustic interferences. Insonation was continued for 3 min at a focal-spot-average intensity of 16 $W/cm^2$. Insonation with second-harmonic superimposition was at a focal-spot-average intensity of 8

W/cm$^2$ at each frequency. The focal spots were electronically moved every eight seconds so as to treat a tumor with seven focal spots with a nearest-neighbor lateral separation of 3 mm. The tumor bearing mice were divided into four groups: untreated, treated with ATX-70 at 0.5 MHz alone, at 1 MHz alone, and at both 0.5 and 1 MHz. Each group consisted of three mice. The tumor volume was estimated form its major and minor axes measured with a slide caliper. The hair over the tumor were shaved before the first measurement.

## RESULTS AND DISCUSSION

Focal damage, typically 3-4 mm in diameter, was produced in the liver tissue by focused ultrasound at certain combinations of the fundamental and the second-harmonic amplitudes depending on the administered sonodynamically active agent. The spot size approximately matches both focal spots of the fundamental and second harmonic. A high erythrocyte concentration and a high proportion of hepatocyte necrosis were observed in its histologic section [8]. The production of the hemorrhagenic tissue damage was always paired with fractional harmonic acoustic emission, which is known to be specific to acoustic cavitation [10]. The temperature rise during insonation reached a plateau within 60 s and was less than 2 °C even at a focal spot average intensity of 24 W/cm$^2$ at 1 MHz. This level of temperature rise is not likely to induce thermal damage of tissues during such a short period of time [11]. These suggest that the observed hemorrhagenic focal tissue damage was likely to be primarily caused by the cavitational rather than thermal effect of ultrasound.

The effect of the fundamental and second-harmonic intensity combinations on producing cavitational tissue damage with and without administration of erythrosin is shown in Fig. 2. The results are plotted with the fundamental and second-harmonic focal-spot average intensities on the horizontal and vertical axes, respectively. Such second-harmonic phase was chosen that the peak rarefaction was emphasized. Synergism between the fundamental and the second harmonic in producing cavitational tissue damage is quite distinctive with erythrosin. Cavitational tissue damage was observed when focal-spot average acoustic intensities at both frequencies was 1 W/cm$^2$ or higher, while a focal-spot average intensity of at least 4 W/cm$^2$ was needed for inducing such tissue damage when only one frequency was used. In the absence of erythrosin, the observed synergism was relatively slight. Erythrosin in combination with second-harmonic superimposition reduced the intensity threshold for cavitationally producing focal damage in mouse liver tissue by more than an order of magnitude. Since the presence of erythrosin in the tissue was so effective to reduce the threshold, it is reasonable to interpret that the tissue damage was produced by sonodynamic events took place in the tissue rather than in degassed saline.

The effect of second-harmonic phase on the intensity threshold for cavitationally producing focal tissue damage with administration of erythrosin derivative was also tested. The intensity ratio between the fundamental and the second harmonic was kept to 1:1. The intensity threshold was significantly low around the second-harmonic phase at which the peak rarefaction was maximized. A similar second-harmonic phase dependence was reported based on *in vitro* experiments [8]. These can be explained by a hypothesis that second-harmonic superimposition enhances cavitational effects through accelerating microbubble growth rather than collapse [6].

The effect of sonodynamic treatment employing second-harmonic superimposition on the growth of experimental murine tumors was also tested. The untreated tumors grew approximately seven times larger in average volume in six days. The effect of single frequency insonation at 0.5 MHz in combination with administration of a Ga-porphyrin, ATX-70, was not significant. The effect at 1 MHz with ATX-70 was significant but only very slight. The effect of second-harmonic superimposition with ATX-70 was significant in comparison with all the other three groups. When the tumors were treated employing second-harmonic superimposition without ATX-70, their difference with the untreated was not significant.

The temporal average of temperature rise in the tumor during insonation was 1 to 5°C. The thermal effect can hardly explain the significant difference between the result with second-harmonic

superimposition and that with 1 MHz alone, because ultrasonic absorption in tissues is higher at 1 MHz than at 0.5 MHz. The significant difference between with and without ATX-70 for the results with second-harmonic superimposition can hardly be attributed to the thermal effect, either. Furthermore, it has been reported that ATX-70 greatly enhanced ultrasonic generation of active oxygen [2]. Therefore, the significant antitumor effect of second-harmonic superimposition with ATX-70 is most likely to have been the result of the sonochemical effect of cavitation efficiently induced through second-harmonic superimposition. Further investigation may be needed for verifying this mechanism. The present experiment was carried out in accordance with the protocol under which one course consists of a single set of treatment though it is expected that further repeated treatment may yield a more remarkable antitumor effect.

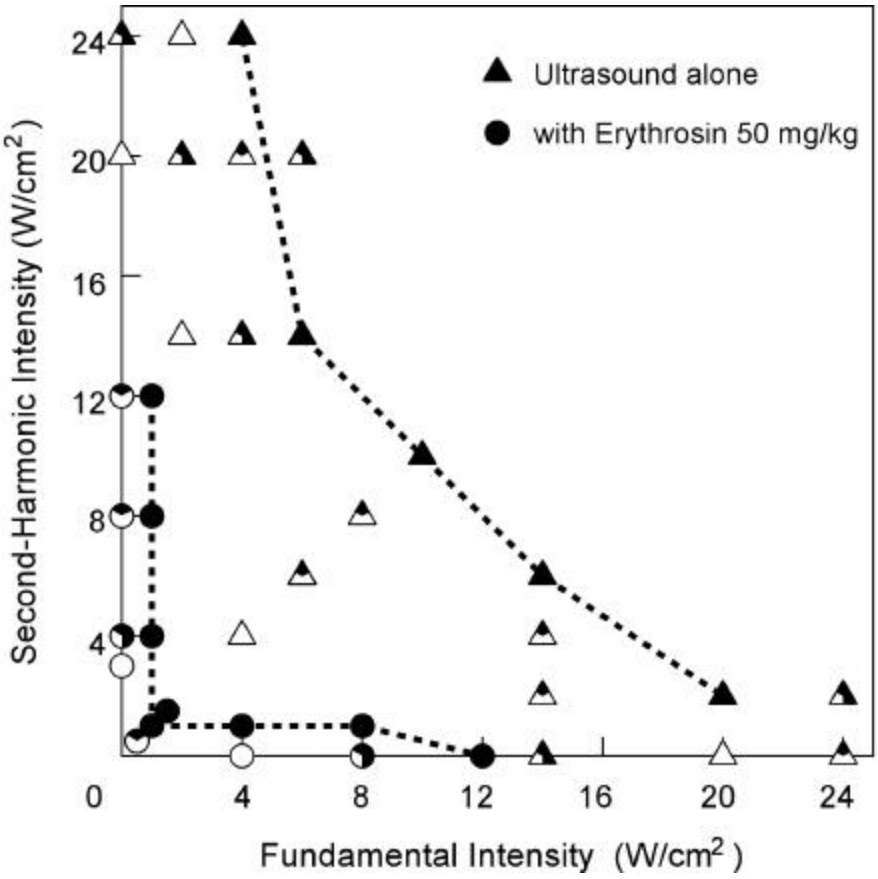


Fig. 1. Enhancement of cavitational production of focal tissue damage in mouse liver by second-harmonic superimposition. The results were plotted with the fundamental (0.5 MHz) and the second-harmonic (1 MHz) focal-spotaverage intensity on each axis. Circles and triangles represent the results with and without administration of erythrosin (50 mg/kg), respectively. Each point represents the result of three mice, and was filled according to the number of mice in which cavitational tissue damage production was observed.

It has already been reported that both certain xanthenes and prophyrines such as erythrosine and ATX-70 show significant sonochemically induced cytotoxicity when they are activated by ultrasound [13,14]. In addition to that, ATX-70 accumulates in tumor tissues at a significantly higher concentration than in normal tissues, and erythrosine significantly reduces cavitation threshold in tissues as shown in Fig. 1. Activation of a tumor selective agent with focused ultrasound will make synergistic targeting in tumor treatment possible. An ideal chemical agent for sonodynamic therapy may have all of these three properties: ultrasonically inducible cytotoxity, tumor selectivity, and the activity to reduce cavitation threshold. However, none of the pre-existing agents have all of them. ATX-70 reduces

cavitation threshold only very slightly, and erythrosine does not accumulate in tumor tissues at a high concentration. In this context, one could naturally conceive two approaches to such ideal agents: superadding tumor selectivity to a certain xanthene and superadding cavitation threshold reducing activity to a certain porphyrine. In the Japanese National Research and Development Program for Medical and Welfare Apparatus, synthesis and evaluation of such potentially ideal sonodynamic agents are under way.

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