Abstract

To improve the effect of ultrasound exposure with second-harmonic superimposition (SHS), which we reported to be effective for inducing cavitational effects, we investigated the effect of periodical shifting of the relative phase on the cavitation induction by using SHS.

In an aqueous solution, subharmonic emission intensity, a measure of cavitation intensity, obtained by using SH phase shifting with a shift pitch not larger than 1/2 p and a shift interval time not shorter than 30 ms was as much as that obtained with the optimum fixed SH phase. When exposed to tumor tissues subcutaneously implanted in mice, SH phase shifting with a shifting period larger than 10 ms was effective to suppress tumor growth, which roughly agree with in vitro results. This result may be important for the application of SHS to in vivo treatment in which it is difficult to maintain the relative phase in a target tissue constant because of movements and biophysical changes.

1. Introduction

Cavitational effects are one of non-thermal ultrasonic bio-effects and may have the highest potential as a selective therapeutic modality among the nonthermal effects of ultrasound, because cavitationally induced short-life intermediates are considered to play an important role in cell lysis [1].

Two major problems of therapeutic application of cavitational effects are that 1) it requires high intensity ultrasound and 2) reproducibility is low without standing wave fields or additional nuclei.

Methods to induce cavitational effects in a progressive wave field and without micron-sized nuclei would widen applicable fields of cavitation. Recently we have found that superimposing the second harmonic onto the fundamental (SHS) can reduce the intensity threshold for cavitation in aqueous buffer solutions [2]. SHS reduced the cavitation threshold by about ten-fold in an experimental setup which minimizes the formation of standing waves. Moreover, SHS was found to reduce the cavitation threshold in living tissues such as mice livers [3]. SHS was most effective for inducing both in vitro and in vivo cavitations when the SH phase is set to emphasize peak rarefaction. Theoretical study on rectified diffusion indicated that SHS accelerates bubble growth in a cavitation process.

Such relative-phase dependence, though, can be a disadvantage in the therapeutic application of SHS when considering that it is not easy to control relative phases in non-uniform media such as the human body.

To develop an SHS method independent to the relative phase, we investigated the effect of periodic shifting of SH phase on the fundamental both in aqueous solutions and mice tumors in this study. Such an exposure method may have an advantage in terms of the efficiency of treatment because a uniform effect of SHS is expected throughout the region to be treated.

2. Methods

2.1. In Vitro Experiments

2.1.1. Experimental Setup

The experimental setup previously described [2] was used. Briefly, an ultrasound transducer 24 mm in diameter and piezoelectrically active at both 1.09 and 2.18 MHz was used. Acoustic emission from 10 % ethyl alcohol (EtOH) was measured with a hydrophone and the temporal average of sub-harmonic component (0.50 MHz) during 2-minute ultrasound exposure (1.09 MHz: 5 W/cm2, 2.18 MHz: 5 W/cm2).

2.1.2. Relative Phase

In this paper, the second-harmonic superimposed signal, \( A_{\text{sh}(t)} \), generated with the arbitrary wave generator is described by

\[
A_{\text{sh}(t)} = A_1 \sin(\omega t) + A_2 \sin(2\omega t + \theta),
\]

where \( A_1 \) is the amplitude of the fundamental wave, \( A_2 \) is the amplitude of the second-harmonic wave, and \( \theta \) is the second-harmonic phase relative to the fundamental. In this paper, \( \theta \) is referred to as the relative phase and used to characterize second-harmonic-superimposed waves. The period for the relative phase to complete a
cycle is referred to as the shift period and the time between
one relative phase shift and the next is referred
to as the shift interval.

2.1.3. Phase Shifting
Shifting was performed by changing the relative phase \( \theta \) at a pitch of either 1, 1/2, 1/4, 1/8 or 1/16.

2.2. In Vivo Experiments
The setup used was the same as that used in the in
vitro section except that ultrasound was exposed at 0.5
and 1.0 MHz (8 W/cm² each). Rose bengal a-
carboxytridecyl ester was injected to anesthetized mice
implanted with colon 26 carcinoma. They were placed
in a water tank filled with degassed water (32 °C) and
ultrasound was exposed for 7 minutes.

3. Results

3.1. In Vitro Experiments
The effect of phase shifting was investigated as a
function of phase-shifting interval. The threshold
interval times required for inducing subharmonic
emission were found to be 3 ms when the shift pitch
was 1/4, 1/8 or 1/16 \( \pi \) and 30 ms when the shift pitch
was 1 or 1/2 \( \pi \).

3.2. In Vivo Experiments
The effect of phase shifting as a function of phase-
shifting interval on colon 26 tumor growth
subcutaneously inoculated onto mice is shown in Fig. 2.
When the shift interval was either 100 or 1000 ms,
tumor growth was smaller than volumes when the shift
interval was 1 and 10 ms.

4. Discussion
Phase shifting of SHS with a shift interval time not
shorter than about 30 ms and a shift pitch not longer
than 1/2 \( \pi \) was found to induce cavitation about as
much as SHS at the optimum fixed relative phase in
aqueous solutions. Such a method of insonation can
induce cavitation independently of the initial relative
phase; thus, it seems to be useful in inducing cavitation
in sites where the relative phase cannot be exactly
controlled such as in the human body. The result on
mice tumors that the shift interval longer than 10 ms
was more effective to suppress tumor growth than
shorter intervals roughly agrees with in vitro results.
Taking the above result and our previous results[2,3]
together with consideration, which showed that the optimum
phases of SHS for inducing cavitation in aqueous
solutions and mouse livers were about the same, we
might expect that the mechanism of how cavitation
was accelerated by phase shifting of SHS in vitro and
in vivo are basically the same.

The result that subharmonic intensity is dependent
on shift interval rather than on shift period indicates
that the length of time the optimum relative phase continues is more important than the number of times
the optimum relative phase appears, thus the beating
the fundamental and a frequency component near the
second-harmonic will not show the same effects as
obtained in this work.

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6. References
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