Behavior of microbubbles in diagnostic and therapeutic ultrasound

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Behavior of microbubbles under exposure to ultrasound has long been discussed in the field of medical ultrasound. Discussion was first focused on ultrasound safety because cavitation bubbles play important roles in this issue. Over the past twenty years, diagnostic applications of microbubbles as ultrasound contrast agents have been discussed, and therapeutic application is now a subject of study that is attracting the interest of many researchers. Since various types of bubbles and exposure conditions of ultrasound have been used in these applications, various types of bubble behaviors have been discussed.

Two types of ultrasound contrast agents, Levovist and Sonazoid, are now available in Japan, and these agents are used at different output settings of Mechanical Index (MI). Levovist is used with a high MI setting because the bubbles are considered to have certain threshold pressures to start oscillation. The bubbles activated by ultrasound of sufficiently high MI start violent oscillation and collapse into small fragments. On the other hand, bubbles of Sonazoid have no threshold pressure to start oscillation and show gentle oscillation under exposure to ultrasound of low MI. Since echo signals from the oscillating bubbles of these contrast agents are different, it is important to use optimized settings of an ultrasound scanner for each contrast agent.

Applications of microbubbles have also been discussed in the field of therapeutic ultrasound. Sonoporation is a technique for introducing drugs or foreign genes into cells by ultrasound exposure, and it is well known that efficiency of sonoporation is greatly improved by exposure of cells to ultrasound in the presence of microbubbles. Generally, sonoporation uses cells and bubbles suspended in a culture medium, and the suspension is exposed to continuous wave (CW) ultrasound. Another type of sonoporation, possible application of which we have been studying, uses cells with an attached microbubble, and the cells are exposed to single-shot pulsed ultrasound. In both methods, addition of bubbles results in a dramatic increase in sonoporation efficiency, but the mechanisms are different. In the case of CW sonoporation, acoustic energy is effectively absorbed by microbubbles of resonant size, and the violently oscillating bubbles collapse into fragments, which later grow to resonant-sized bubbles by rectified diffusion. Since this process is repeated many times under exposure to CW ultrasound, addition of microbubbles causes production of a large number of inertial cavitation bubbles, resulting in increase in the efficiency of sonoporation. In the case of pulsed wave sonoporation, addition of bubbles does not increase the number of cavitation bubbles. In this method, however, the presence of bubbles adjacent to cells increases the efficiency of bubble behavior to cause damage to the cell membrane. Production of small jets of surrounding liquid during nonuniform contraction of a bubble is the possible mechanism for producing membrane damage.

An understanding of bubble behavior and its effects on cells is essential to determine suitable exposure conditions of bubbles for diagnostic or therapeutic applications. Further efforts should be made to elucidate the behavior of microbubbles in the field of medical ultrasound.
Molecular Therapy Using Ultrasound: Mechanisms Involved in Drug Activation, Apoptosis Induction, Gene Transfer, and Alterations of Gene Expression

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Interest in molecular imaging and in molecularly-targeted therapy has grown tremendously, and ultrasound may offer new tools for modern cancer therapy. To understand how therapeutic ultrasound works, it is necessary to understand its biological effects at the molecular level. In this review, investigations on the molecular aspects of ultrasound are discussed, with emphasis on apoptosis induction, gene expression, and gene transfection. In these studies, apoptosis induction was assayed with flow cytometry and with other methods targeting indicators of apoptosis. Gene expression was evaluated using western blotting, real-time polymerase chain reaction, and microarray analysis. Gene transfection was investigated using a luciferase assay and other methods. The reported results show that low intensity ultrasound can induce apoptosis in cancer cell lines, and that this effect can be optimized using pulsed ultrasound. Sonication can result in the down-regulation or up-regulation of some genes. Of particular interest is the striking up-regulation of the heme oxygenase-1 gene, a gene usually associated with oxidative stress in human lymphoma U937 cells. Introducing genes using ultrasound with or without microbubbles also exhibited promising results. Membrane damage is pivotal to biological effects, and using ultrasound to modify cell membranes can either promote or inhibit desired effects. In summary, it is concluded that ultrasound has the potential to help develop useful methods which can be utilized in therapies which require apoptosis induction, gene introduction into cells, alterations in gene regulation, and drug-activation.

References
Non-invasive therapy with high-intensity focused ultrasound (HIFU) has been successfully applied to transrectal treatment of prostate cancer etc. However, its clinical application is still limited due to its low treatment throughput. Microbubbles are known to enhance the mechanical and sonochemical bioeffects of ultrasound, whether they are administered into the body as a stabilized form or ultrasonically created in situ. Recently, they were reported to also enhance the thermal bioeffect of ultrasound and to have a potential significantly accelerating HIFU treatment thereby. Two major mechanisms have been proposed for a microbubble to convert the acoustic energy into heat: viscous heating and a pressure-volume (p-V) hysteresis loop. A microbubble in an ultrasonic field increases the shear displacement in the surrounding medium by orders of magnitude, resulting in heat generation due to the viscosity of the medium. A microbubble subjected to acoustic pressure is exposed to a thermal cycle. Its p-V hysteresis loop is negligible when the cycle is nearly either isothermal or adiabatic, but heat equal to the area of the loop is generated per cycle when the cycle is similar to neither of them. When the gas species inside the microbubble is polyatomic as those of microbubble agents in the second and third generation, the thermal cycle is nearly isothermal and the heat generation due to its p-V hysteresis loop is negligibly small.

The ultrasonic power converted into heat by a microbubble through viscous heating was calculated by numerically solving a modified Rayleigh-Plesset equation. At an ultrasonic intensity of 1 W/cm² and a frequency of 3 MHz, a resonant microbubble, approximately 1 µm in radius, converted ultrasonic power in the order of 10 µW into heat. It is estimated that the ultrasonic heat generation in tissue will be doubled if such a microbubble agent is delivered to the tissue at a concentration in the order of 10 microbubbles/mm³, which is normally within the safe dose range approved for the diagnostic use of a microbubble agent. An exteriorized murine kidney was exposed to focused ultrasound at 3.2 MHz in degassed saline and the tissue temperature change was measured. The ultrasonic intensity was carefully chosen to a few hundred W/cm², which is lower than a typical intensity for HIFU by an order of magnitude, for repetitive measurement so that the tissue temperature did not exceed the coagulation level. With an intravenous bolus administration of 0.2 ml/kg Optison™, the ultrasonically induced temperature elevation was multiplied by up to five times. The enhancement in temperature elevation gradually decreased as the microbubble agent was eliminated from the body. The experimental results agreed with the theoretical prediction in the order of magnitude.

The predicted and observed effect of a microbubble agent may have a potential use to enhance HIFU treatment. However, the significant portion of the ultrasonic power will be converted into heat in the intervening tissues before delivered to the tissue to be treated if the microbubbles are distributed rather uniformly in the body. Therefore, the microbubbles have to be somehow selectively delivered to the tissue to be treated in order to make this approach really useful. Tissue-targeted microbubbles coated with certain ligands may be a potential vehicle for the selective delivery. Another potential vehicle may be nano-droplets convertible to microbubbles, which are designed not to be converted with HIFU but to be with a special sequence of focused ultrasound with much lower energy than HIFU.
Angiogenesis and Ultrasound
-Anti-Angiogenesis Therapy in Combination with Ultrasound Irradiation for Human Cancer Evaluated by Contrast Color Doppler Ultrasound-

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Abstract
Angiogenesis, the growth of new capillary blood vessels from pre-existing vasculature, is a crucial process for tumor progression and metastasis. The microvascular endothelial cells (ECs), which are recruited by tumors, have become an important target in cancer therapy. Angiogenesis inhibitors have thus been developed to target vascular ECs and block tumor angiogenesis. Ultrasound has been shown to enhance the anti-tumor effect of a chemotherapeutic agent in vitro and in vivo. Transiently increased permeability of cell membrane is one of the mechanisms of the US-enhanced chemotherapy. Sonoporation, and resealing of cell membrane by acoustic pressure are considered to be a primary reason for an increased intracytoplasmic concentration of the administered agent. Our study firstly examined the antitumor effect of angiogenesis inhibitor combined with US irradiation for human cancer in vivo and evaluated its vascularity by color Doppler US in real time using a microbubble US contrast agent (Emoto M, Tachibana K, et al. Cancer Science 98:929-935, 2007). In summary, a human uterine sarcoma cell line, FU-MMT-1 (Emoto M, et al. Cancer 1992), was used in vivo because this tumor is one of the most malignant neoplasm of the human solid tumors and it also has a poor response to any of the chemotherapeutic agents currently used as well as to radiotherapy. The FU-MMT-1 xenografts in nude mice were treated by US at a low-intensity (2.0 w/cm², 1MHZ) for 4 min three times per week each after the subcutaneous injection of TNP-470 (30 mg/kg), an angiogenesis inhibitor, and this treatment was continued for eight weeks. Either treatment of US alone or TNP-470 alone showed a suppression of tumor growth, in comparison to the non-treatment group (control), and a significantly enhanced effect was obtained by the combined treatment. A reduction in the intratumoral vascularity, which was evaluated by both enhanced color Doppler and immunohistochemistry, was significantly demonstrated by the combined treatment, in comparison to each treatment alone, and the control (Figures). No side effect was observed in any mice in the combined treatment group. In conclusions, these results suggest that anti-tumor effect of TNP-470 for uterine sarcoma was accelerated by US irradiation in vivo and this combination might be a potentially effective for new cancer therapy. Color Doppler US with microbubble contrast agents can non-invasively evaluate the effects of any cancer treatments in real-time.

Keywords: angiogenesis, anti-angiogenic therapy, TNP-470, ultrasound irradiation, contrasted color Doppler ultrasound

References
