Sound Waves and Antineoplastic Drugs: The Possibility of an Enhanced Combined Anticancer Therapy
Loreto B FERIL, Jr, Takashi KONDO, Shin-ichiro UMEMURA
Katsuro TACHIBANA, Angelo H MANALO, and Peter RIESZ

Abstract
Kremkau wrote a historical review of the use of ultrasound in cancer therapy in 1979. In 1990, Kondo and Kano published a Japanese review of the implications of the thermal and nonthermal effects of ultrasound in the treatment of cancer. Again in 2000, Kondo et al reviewed the therapeutic applications of ultrasound and shock wave, emphasizing their thermal and cavitation effects. Here we focus on the effects of ultrasound or shock waves in combination with anticancer agents, emphasizing their mechanisms of action and interaction. Most of the studies cited here reported promising results. Although the extent of the augmented combined effects in vivo is limited, synergism is the rule in vitro. In addition to the thermal effect of ultrasound, cavitation effects undoubtedly played a major role in both ultrasound and, more prominently, in shock wave therapy. Although the mechanism of the nonthermal noncavitational effects on biological processes is obscure, several factors, including temperature and the occurrence of cavitation and inertial cavitation, probably coexist and blend with these other effects. Magnification of anticancer activity results mainly from increased localization of drugs or other agents in vivo and increased intracellular permeabilisation both in vivo and in vitro. On the other hand, sublethal damage caused by ultrasound or shock waves may render cells more susceptible to the effects of the agents, and both may act together, further amplifying these effects. We thus conclude that proper combination of an appropriate agent and ultrasound or shock wave should help improve cancer therapy by minimizing the side effects of drugs by lowering the effective dose and reducing the systemic concentration while increasing the efficiency of the therapy as a whole. Future studies should reveal specific conditions in this combined therapy that will lead to optimal outcome.


Keywords
anti-cancer agent, combined anticancer therapy, shock wave, ultrasound

1. Introduction
Newton characterized the physical nature of sound as a form of mechanical energy in the 17th century. Because sound is the main input for hearing, Helmholtz, in the 19th century, explored the physiology of hearing. Then, in the early 20th century, the unheard aspect of sound came into practical use. This was the advent of vacuum-tube technology and the principle based on piezoelectricity, which could generate sound of very high frequency. Sound navigation and ranging (SONAR) proved useful, and ultrasound became important in diagnostic medicine in the late 1940s. Since then, ultrasound-defined as sound too high in frequency to be detected by the human ear, usually at frequencies greater than 30 kHz, has become an increasingly important tool in medical science. At the other end of the spectrum of sound, shock wave, often mistakenly considered to be a form of ultrasound, has also found unique clinical applications in recent years.

Ultrasound is widely used for soft tissue imaging. Its use became common because of its perceived

1Department of Radiological Sciences Toyama Medical and Pharmaceutical University, Toyama 930-0194, Japan, 1Hitachi, Ltd Central Research Laboratory, Higashi-koigakubo, Kokubunji, Tokyo 185-8601, Japan, 1First Department of Anatomy Fukuoka University School of Medicine, Fukuoka 814-0180, Japan, 1College of Medicine Mindanao State University, MSU-IIT, Iligan City, Philippines, 1Radiation Biology Branch National Cancer Institute, NIH, Bethesda, MD 20892-1002, USA
Recieved on February 25, 2002; Accepted on May 17, 2002
safety, noninvasiveness, and low cost. It has also been used therapeutically in surgery, ophthalmology, physical therapy, and cancer therapy. Shock wave therapy is used mainly in lithotripsy. Pioneering reports concerning the use of ultrasound in the treatment of malignancies were published in 1930s\(^5\). Most of the early work was carried out in Japan\(^5\).

Because of its sufficient tissue attenuation coefficient and easy, manageable focusing, ultrasound has been studied extensively for use in hyperthermia for cancer therapy and in tissue ablation using high-intensity focused ultrasound\(^6\). Therapeutic use of such nonthermal effects as cavitation is also gaining attention in cancer research. Acoustic cavitation, ultrasonically induced cavitation, is known to be the primary cause of sonoluminescence, mechanical shock waves, and sonochemical reactions producing reactive oxygen species\(^7\). The principle of mechanical disturbance brought about by cavitation produced by ultrasound is similar to that caused by shock waves. Together with cavitation, other ultrasonic effects that are thought to be nonthermal and noncavitational\(^9\) offer greater potential in cancer therapy, especially when they are combined with the use of other agents. This is pointed out by some of the studies reviewed here. In the review that follows, we discuss various aspects of this treatment modality, placing particular emphasis on the mechanism of action by which anticancer agents act on cells when combined with exposure to ultrasound (US) or shock wave (SW).

2. Ultrasound in Combination With Anticancer Agents

The combination of therapeutic ultrasound and other modalities has been investigated for several decades, especially in the treatment of cancer\(^1\). Here we discuss the combination of ultrasound with chemotherapy (Table 1).

2.1 Studies in vitro

Ultrasound (frequency, 2.025 MHz; intensity, 0.5 to 2.0 W/cm\(^2\)) was used in combination with four different drugs: adriamycin, amphotericin B, cisplatin (both at 1 \(\mu\)g/ml), and etoposide (10 \(\mu\)g/ml)\(^6\). Although the effects of adriamycin and amphotericin B on Chinese hamster ovary cells (HA 1) were enhanced, the effects of cisplatin and etoposide were not. Increased cytotoxicity was observed only at hyperthermic temperatures and lower intensities of ultrasound, but the cytotoxic effect was also observed at higher intensities at 37°C. Enhancement was more prominent with adriamycin and this might have been due to increased cellular uptake of the drug, altered sensitivity of the cells, or potentiation of the molecular activity of the drug, alone or in combination. Successive studies that focused on the use of adriamycin showed promising results. Ultrasound (2.6 MHz cw at 2 to 3 W/cm\(^2\)) has rendered adriamycin (1 to 8 \(\mu\)M) more toxic as measured by delay in the growth of fibroblasts from the lungs of Chinese hamsters (V 79-379 A)\(^10\). This effect, though uncertain, may be attributed to bulk-heating effects and increased intracellular concentration of the drug as measured by flow cytometry. By contrast, the effects of adriamycin were not significantly enhanced\(^12\) in a study using 1.62 or 1.765 MHz ultrasound at intensities of 1 to 2.5 W/cm\(^2\). The authors conclude, however, that synergy of ultrasound with adriamycin is more likely than with hyperthermia or ionizing radiation. In another study, adriamycin at 40 to 160 \(\mu\)M, caused no cell damage in sarcoma 180 cells\(^13\). When combined with ultrasound at 1.93 MHz, 6 W/cm\(^2\) progressive mode, the effect was three times greater than that obtained with ultrasound alone. This result is closely correlated with the detected generation of nitroxide. Synergistic loss of cell viability was prevented in the presence of 10 mM histidine, but not in the presence of 100 mM mannitol, suggesting that singlet oxygen mediated the sonodynamic effects. A less cardiotoxic adriamycin derivative, 4′-O-tetrahydro-pyranyladrioniamycine (THP-adriamycin), produced similar augmentation in sarcoma 180 cells at 16, 40, or 80 \(\mu\)M, and the same singlet oxygen-mediated mechanism was proposed\(^14\). Another drug, AraC, showed synergism with ultrasound (48 kHz at 0.3 W/cm\(^2\)) on the clonogenicity of HL-60 cells. Increased uptake of the drug as a result of ultrasound-induced alteration of the cell membrane was suggested as a cause\(^15\). An effect of ultrasound in combination with vincristine, an antineoplastic alkaloid, on seedling roots of Zea maize was also investigated and found to act independently, and no synergism was observed\(^16\).

Some agents that lack known anticancer effects or cellular toxicity became active when used in combination with ultrasound. One such agent is ATX-70, a gallium-porphyrin analogue that was up to four times more effective in killing sarcoma 180 cells than ultrasound (1.93 MHz, 4.5 W/cm\(^2\)) alone\(^17\). Using the same ultrasound setup, a synergistic effect on the same cell line was also observed with hematoporphyrin (Hp) at 50 \(\mu\)g/ml\(^18\). In these studies, the mechanism of singlet oxygen was considered because of the blocking effect of histidine, a known \(^{1}O_2\) and OH-scavenger, and further supported by the increased effect produced when D\(_2\)O, which is known to prolong the life-span of singlet oxygen, was used instead of H\(_2\)O in the medium. However, these findings did not agree with results obtained using ultrasound (50 kHz) in combination with ATX-70, which showed enhanced toxicity on human leukemia HL-525 cells but did not detect radical intermediates by EPR spin trapping or block the effects when 70 mM POBN was used\(^19\). Photofrin II (porfimer sodium), on the other hand, already