# A review of research into the uses of low level ultrasound in cancer therapy

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## Abstract

The use of low power ultrasound in therapeutic medicine is a developing field and this review will concentrate on the applications of this technology in cancer therapy. The effects of low power ultrasound have been evaluated in terms of the biological changes induced in the structure and function of tissue. The main fields of study have been in sonodynamic therapy, improving chemotherapy, gene therapy and apoptosis therapy. The range of ultrasonic power levels that can be effectively employed in therapy appears to be narrow and this may have hindered past research in the applications in cancer treatment.
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## 1. Introduction

Therapeutic ultrasound is defined as the use of ultrasound for the treatment of diseased organs or structures. This field is continuously expanding and new clinical applications are being developed constantly. Such clinical advances have been made possible by a number of factors including advances in transducer design technology, laboratory experiments to determine the precise chemical reactions taking place during or following exposure to ultrasound and advances in measurement and calibration of acoustic power for the safe application of ultrasound in therapy. Somewhat surprisingly, given some quite remarkable laboratory results, progress in the wider clinical use of ultrasound for therapy has been very slow except in some well-defined fields such as extracorporeal lithotripsy, physiotherapy, ultrasonic surgical instruments and, more recently, high intensity focused ultrasound (HIFU).

Throughout this review low power (or low level) ultrasound refers to the acoustic energy delivered by the transducer. The energy may be applied over a range of frequencies that can be generally grouped into those shown in Fig. 1 but are mostly in the so-called physiotherapy region.

Biological effects of ultrasound and their applications are a rapidly expanding research area. In recent years ultrasonic therapy for tumours has been developed successfully. HIFU has been used to treat solid tumours and its efficacy and safety have been confirmed in clinical investigations [1]. HIFU can be carried out either as a radical surgery or as a palliative therapy. According to some reports in the literature, some patients with inoperable liver masses are still alive and free of tumour 24 months after receiving HIFU therapy [2,3]. On the other hand, researches into the bioeffects of relatively low-intensity ultrasound on malignant tissues and their applications are still in the process of investigation. The use of this type of low-intensity ultrasound-therapy has great potential in that it can be relatively easily applied.

There have been a series of investigations which appear to show that the responses of malignant cells to low-intensity ultrasound are not identical to those of normal cells in that cancer cells were more prone to being killed [4,5]. Low-intensity ultrasound can also suppress cell proliferation and clone formation, improve the effects of anticancer chemicals and deactivate cells via indirect mechanisms [4–7]. These findings revealed

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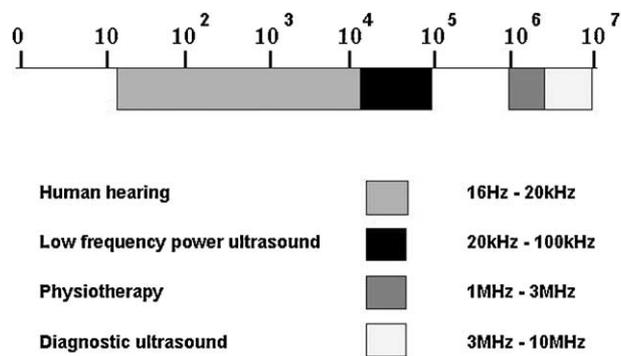*E-mail address:* yutinghe@hotmail.com (T. Yu).

Fig. 1. General classification of the frequency ranges of ultrasound.



Fig. 2. Illustration of analysis of bioeffects of ultrasound and their applications from perspectives of tissue structure and function. $L_I$: the critical level for injury; $L_D$: the critical level for death. (I) Therapy for tumor and tumor-like lesion; (II) gene expression, molecular delivery and fracture healing, etc. Solid curve: structure, dotted curve: function. First published in "Biological effects of ultrasound exposure on adriamycin-resistant and cisplatin-resistant human ovarian carcinoma cell lines in vitro (T. Yu et al., Ultrasonics Sonochemistry, this issue), with permission from Elsevier B.V.

that low-intensity ultrasound has distinct potential as a technique for cancer treatment.

## 2. Evaluating bioeffects from the perspective of tissue structure and function

The bioeffects normally associated with exposure to ultrasound are heat, mechanical effects and acoustic cavitation. However, these three mechanisms do not function in the same way. Bioeffects are also intensity- and frequency-dependent. A higher intensity benefits heat-production, and a lower frequency favours the occurrence of cavitation. Therefore the acoustic parameters must be selected carefully when using ultrasound in therapy according to the objective required.

The exposure of biological tissues to ultrasound can result in structural and/or functional alterations. Structural changes range from slight but repairable damage to immediate death. The functional alterations include proliferation, migration, synthesis, secretion, gene expression and membranous action, etc. [4,6,8,9]. On most occasions, structural changes in tissue brought about functional alterations and vice versa. Occasionally only functional alterations were detected in cases where the structure change was too small to be identified.

From the literature and our own work, we believe that the bioeffects of ultrasound and their applications can be analyzed from the perspective of tissue structure and function. The sonication "level" was determined by intensity, frequency and exposure duration, etc. There are two critical levels in respect of tissue structure, one relating to the onset of cell damage ($L_I$) and the other to cell death ($L_D$). Morphological changes occur when tissues are exposed to an insonation above $L_I$. Cells would be immediately deactivated if the ultrasound level was $\geqslant L_D$. Biological effects can also be understood from the perspective of tissue function. The sonication acts as either an activator or an inhibitor. Variations of structure and function with the elevation of the sonication level are illustrated in Fig. 2. The functional change is
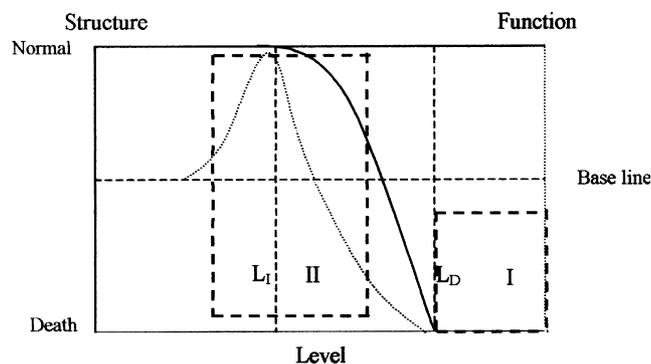
biphasic and the structural alteration monophasic. Both structure and function are affected when tissues are exposed to levels just above $L_I$. Therefore, it is important to achieve a balance between structural and functional changes.

Biomedical applications of ultrasound can also be evaluated from the perspective of structure and function. We have divided these applications of ultrasound into two groups; one to mainly induce structural alteration and the other to mostly modulate function. Levels above $L_D$ resulted in immediate cell death, so they can be used to destroy tumours and tumour-like lesions, such as warts and benign prostate hypertrophy. These applications are included in the group that induces structural alterations. Levels around $L_I$ mainly induce functional changes; therefore they are ideal approaches to modulate tissue functions, such as gene expression and protein synthesis. Ultrasound at this level deactivates tissues via an indirect mechanism.

In previous studies, the ultrasonic "level" was determined by the intensity and an intensity of 3 W/cm$^2$ (or sometimes 2 W/cm$^2$) was regarded as the critical value between low-level and high-level ultrasound. We believed that it was more reasonable to distinguish such levels from the bioeffects produced. This was because, (1) applications were based upon bioeffects, (2) the intensity in vivo was affected by lots of factors (tissue type, functional status and exogenous factors, etc.). Intensities observed in situ were dramatically different despite an identical applied acoustic intensity. As a result, there are great differences in the responses in tissue, ranging from zero reaction to complete deactivation. Furthermore, therapeutic ultrasound is usually considered to operate in the range of non-linear acoustics [10], resulting in difficulties in predicting ultrasonic behaviour in tissue.

We suggest that ultrasound waves, which mostly induce structural alterations, are high-level. On the other hand, those that mainly modulate tissue functions are low-level.

Despite the fact that the use of low-level ultrasound in therapy can be easily administered it has lagged behind the use of high-level ultrasound. We believe that this is because it is a two edged sword, in that it has both positive and negative effects. It can suppress the mitosis of cancer cells benefiting treatment; however, it could also trigger the proliferation of malignant tissues thus contributing to spreading and metastasis. This suggests that the acoustic parameters and the exposure approaches must be determined strategically while treating malignancies with low-level ultrasound. Only in this way can the maximum therapeutic effects be realized, and the side effects minimized.

## 3. Sonodynamic therapy

Sonodynamic therapy (SDT) is related to photodynamic therapy (PDT), in which therapeutic effects can be mediated by free radicals. Some chemicals produce free radicals on irradiation with light, especially laser. These active molecules destroy biological tissues, thus producing therapeutic effects and PDT has been adopted in clinical cancer therapy. Investigators have found that ultrasound exposure can play a similar role and this has been termed SDT. SDT results from the non-thermal effects of ultrasound, especially cavitation [11].

Ultrasonic cavitation generates free radicals from the breakdown of water molecules. The initial step in the decomposition of water is the production of hydrogen and hydroxyl radicals. Other species, such as hydrogen peroxide, singlet oxygen and superoxide ions, are formed later depending on the specific conditions [12]. Hydrogen superoxide is a very reactive molecule, which can directly deactivate large molecules, such as proteins and nucleic acid. It can also lead to the generation of other free radicals with extensive bioactivities. For these reasons, hydrogen superoxide might be thought of as an amplifier for the production of active ions. Other chemicals can be chemically activated by exposure to ultrasound, resulting in the production of a large number of active ions. These chemicals are known as sonosensitizers. A series of in vitro and in vivo trials confirmed that SDT, in which either the ultrasound or the chemical had no or very low cytotoxicity, could efficiently destroy malignant cells/tissues [13–15]. Ultrasound waves could be precisely focused on the target volume, which made it possible to control the generation of active radicals in a definite area, so only preselected tissues were damaged. This indicated that SDT has the potential for targeted therapy.

The majority of sonosensitizers are porphyrins and their derivatives. Hematoporphyrin, pheophorbide A, photofrin, photofrin II, ATX-70 and ATX-S10 have been used in SDT [13–20]. Other compounds, such as merocyanine 540, erythrosin B and dimethylformamide, can also be chemically activated by sonication [21,22]. Non-steroidal anti-inflammatory drugs, tenoxicam and piroxicam, can be used in SDT [23,24]. In vitro investigations showed that SDT led to cell lysis in erythrocytes, sarcoma 180, L1210 and HL-60 and others [13–15]. Animal experiments suggested that this therapy was effective in treating sarcoma 180 and colon 26 carcinoma [15,18–20]. Umemura et al. compared the effect of hematoporphyrin with that of ATX-70 in sarcoma 180 in vitro [16]. It was possible to double cell kill with hematoporphyrin at a concentration of 80 µM using ultrasound (4.5 W/cm$^2$, 1.93 MHz) but it was improved by a factor of 3 using ATX-70 under the same conditions. This finding suggests that the structure of the sonosensitizer has an impact on the efficiency of SDT.

Investigations into the mechanism of SDT revealed that active oxygen, especially singlet oxygen, was the mediator in the porphyrin- and non-steroid chemical-induced SDT. Its efficiency could be blocked by histidine, the scavenger for singlet oxygen [16,17,19,23,24]. Investigators found that free radical scavengers and antioxidants, such as mannitol, vitamin C, vitamin E and superoxide dismutase (SOD), could reduce cavitation-induced tissue damage [25–27]. These chemicals could be used to protect normal tissues from being destroyed.

The pharmacokinetics of porphyrin have been investigated. In order to destroy target malignant tissue and reduce the poisoning of normal cells, insonation was administered at the specific time when the ratio of concentration of the chemical in the cancerous material to that in plasma reached a maximum. By this method, the therapeutic effects were realized satisfactorily and side effects on normal tissues reduced [15,18]. However, the tissue distribution of sonosensitizers was agent-dependent and this gave rise to other problems:

(1) The therapeutic concentration of the chemical may not be reached in a specific tissue.
(2) The concentration gradient between malignant tissue and its adjacent normal tissue may not be high enough to carry out SDT safely.
(3) The concentration in the target tissue could be lower than that in plasma, resulting in hemolysis and blood cell rupture.

We believe that endoscopic ultrasound and/or intracatheter ultrasound are probably the key, by which the ultrasonic energy can be delivered directly to the malignant lesion. Exploring a series of sonosensitizers with specific pharmacokinetics characteristics makes it

possible to select a specific agent for a specific tissue or organ. This provides an effective approach for SDT.

Jin et al. reported a treatment of murine skin squamous cell carcinoma using a combination of PDT and SDT. The medial survival period in animals receiving combination therapy (>120 day) was longer than that in mice receiving only PDT or SDT (77–95 days). Pathological examinations revealed that the combination of SDT and PDT induced tumour necrosis more extensively [28].

Another advance in SDT was provided by the introduction of antibodies. An antibody was coupled with the sonosensitizer, so that they could link to target cell membrane specifically and efficiently. During insonation the target cells were destroyed efficiently. This technique made therapy more precise [29]. This approach shows great promise for the improvement of antibody-directed target therapy.

## 4. Enhancing chemotherapy

Chemotherapy plays a very important role in cancer treatment however the application of anticancer agents is hampered by their adverse effect on normal tissues. Oncologists have focused on enhancing malignant cells destruction while at the same time reducing side effects. Unfortunately the development of drug-resistance has also contributed to the failure of some treatments.

Ultrasound exposure can enhance the cytotoxicity of anticancer chemicals to cancer cells in vitro. If the same concentration of cytotoxic agents are used, more cells are killed if sonication is applied. This has made it possible to lower the dosage while maintaining or even improving the therapeutic efficiency. As a result, the patient's tolerance to chemotherapy is ameliorated.

Researchers have shown that sonication can synergize the effects of adriamycin, cisplatin, 5-fluorouracil (5-FU), arabinosyl cytosine (Ara C), cisplatin, boron compound HB (dihydroxy (oxybiguanido) boron (III) hydrochloride monohydrate), diaziquone and $4'$-O-tetrahydropyranyladriamycin (THP) [7,30–34]. The synergism has been confirmed in cells with tissue types of ovarian cancer, breast cancer, cervical cancer, leukaemia, Swiss ascites tumour and fibroblast [7,31–33]. Either continuous wave or pulsed wave (including toneburst ultrasound) can be used as the sensitizer [7,32]. We found that the sequence of administering cytotoxic agents and sonication had an impact on the therapeutic efficiency. If adriamycin was given prior to ultrasound exposure, the cell survival rates were lower than those obtained when insonation was performed before adriamycin administration [7].

In the in vitro experiments, cells were suspended in liquids and the mixture was then exposed to ultrasound. This induced cavitation in the liquid i.e. extracellular cavitation. Extracellular cavitation can be detected instrumentally and is capable of rupturing cell membranes. However, the threshold for cavitation in vivo is much higher than in vitro. Further it is difficult to detect cavitation in vivo and to distinguish intracellular cavitation from extracellular cavitation. These factors make the investigations of the effects of ultrasound in vivo difficult to characterise. Investigations of tumours in animals confirm that there is a synergism between anticancer drugs and ultrasound exposure in vivo. The co-administration of anticancer agents and ultrasound suppressed tumours more significantly than drugs alone and ultrasound in the absence of drugs had very limited antitumour activity. Examples of drugs which could be efficiently synergized by ultrasound exposure in vivo are adriamycin, 5-FU, HB, Ara C and bleomycin [31,33, 35,36].

Although ultrasound-induced heat was a stimulator of membrane permeability, many investigators believe that cavitation is the mechanism of the synergism between anticancer drugs and low-level sonication. Free radicals generated by acoustic cavitation can damage cell membranes resulting in the promotion of membrane permeability. This improves the trans-membrane transportation of drug molecules resulting in an increase in intracellular drug accumulation. Support for a nonthermal effect as the mechanism for the ultrasound-induced synergism comes from the absence of detectable temperature-rise in many investigations.

There were no unanimous conclusions about structural changes in the cell membrane although its permeability was increased by ultrasound. Indeed many researchers believe that no significant structural changes occur. Saito et al. reported that ultrasound-permeated corneal cells could not be morphologically distinguished from those unaffected cells [37]. However, Tachibana et al. found that sonication resulted in a reduction of microvilli and membranous laminar ruffles and even membrane pore formation in HL-60 cells [21]. We have investigated, by transmission electron microscopy, human ovarian carcinoma cells exposed to ultrasound which enhanced the cytotoxicity of adriamycin. Only swollen mitochondria and cytoplasmic vacuoles were detected. The cytoplasmic vacuoles are usually regarded as direct evidence of cavitation.

The interaction between ultrasound exposure and microcapsulated adriamycin has been investigated in recent years [38–40]. The $IC_{50}$ observed for free adriamycin and co-polymer micelle P-105 were 1.25 and 2.25 µg/ml respectively, which were decreased to 0.9 and 0.19 µg/ml under sonication [38]. Similar findings were found for paramagnetic analogue ruboxyl [39], suggesting that the form of preparation affected the synergism resulting from insonation.

The ultrasonically induced increase in intracellular drug accumulation cannot explain all the synergistic

Table 1
Values of $D_0$ and $N$ in three groups while adopting single-hit, multi-target model

| Group | $D_0$ (μg/ml) | Ratio | $N$ | Ratio |
|-------|---------------|-------|-----|-------|
| ADR | $5.8556 \times 10^{-2}$ | | $5.2999 \times 10^{-1}$ | |
| US + ADR | $4.2061 \times 10^{-2}$ | 0.7183 | $2.9080 \times 10^{-1}$ | 0.5487 |
| ADR + US | $6.8515 \times 10^{-2}$ | 1.1701 | $1.3180 \times 10^{-1}$ | 0.2487 |

Single-hit, multi-target model: $S = 1 - [1 - \exp(-D/D_0)]^N$, $S$: survival rate; $D$: dosage.

effects. We have evaluated the dosage-response curve of human ovarian carcinoma cell line 3AO exposed to adriamycin and ultrasound using a radiation biology approach, because the rate of cell-kill by anticancer chemicals followed first-order kinetics [41]. The single-hit, multi-target model was used to fit the curves, and $D_0$ and $N$ were used to reflect the effects of ultrasound exposure (Table 1).

Cells were exposed to adriamycin only in group ADR, to adriamycin prior to sonication in group ADR + US and to the anticancer drug following ultrasound exposure in group US + ADR. The synergism occurred in both group US + ADR and group ADR + US. Survival rates in group ADR + US were lower than those in group US + ADR. The group ADR was used as the reference for calculating the ratio.

These findings suggest that exposure to ultrasound alter the intrinsic parameters of the cells, resulting in a shift of response to other stimuli. Sonication, which alone has zero or very slight cytotoxicity, lowered the threshold of cell deactivation. The results also revealed that the method of insonation has an impact on the final effects of ultrasound. We showed that ultrasound-induced synergism also worked in human ovarian carcinoma adriamycin-resistant cells and that the reversal attributable to verapamil could be enhanced by sonication [42].

Ultrasonically induced collapse of microspheres can be used in the control release of drugs. Thus if anticancer chemicals are encapsulated in microspheres they can be transported to the target organ via circulation, then ultrasound can be used to induce their collapse to release the drugs. Using this technique, cytotoxic drug molecules have been targeted on malignant lesions directly and efficiently. In such cases, the collapse of cavitation bubbles leads not only to the release of drugs but also the permeabilization of surrounding cells/tissues (vessel endothelium, basal membrane). This could assist in the trans-barrier transportation of the drug, such as the blood-brain barrier and blood-testes barrier (Fig. 3).

The ultrasonically induced permeability was found to be intensity and exposure-duration dependent, and the effect was transient as long as the cell was not deactivated. Accordingly, the permeability can be adjusted to lead to maximum beneficial effects and investigators
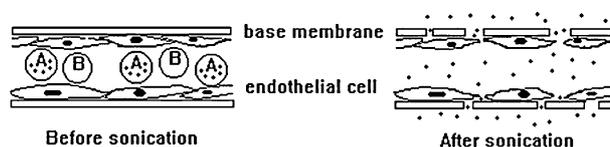


Fig. 3. Illustration of target release of anticancer agents by co-administration of cavitation bubble collapse and microencapsulated chemicals. (A) Microencapsulated chemical; (B) cavitation bubble.

have tried to quantify the permeability according to acoustic parameters. Liu et al. believe that the permeability is controlled by acoustic pressure at 1/2 driving frequency and its ultraharmonics [43]. As the bioeffects are tissue-dependent, an identical acoustic parameter resulted in various changes among different tissues. This suggested that estimating permeability based only upon ultrasonic characteristics is not an adequate approach.

## 5. Gene therapy

Gene therapy is regarded as a very promising technique by which malignancies could be cured radically. However, thus far no satisfactory effects have been found in clinical trials concerning cancer treatment using gene therapy [44].

Two problems must be solved in gene therapy: (a) the transfer of a target DNA sequence and (b) the control of expression of the therapeutic genes transferred. Therapeutic effects can only be attained when adequate genes are transferred into target cells. For safety reasons, it is necessary to ensure that the target DNA will express within a specific range and within a specific tissue/organ. In other words, the expression level of the gene must also be kept controlled so as not to affect the normal physiological functions of tissues once the curative effects are realized.

Ultrasound exposure can be used to improve transfection efficiency. Tata et al. transferred a plasmid encoding GFP into prostate cancer cell line LnCap using ultrasound. Both continuous wave (932.7 kHz, $I_{SATP}$ 1.67 W/cm$^2$) and tone-burst ultrasound ($I_{SATA}$ 0.33 W/cm$^2$, $I_{SATP}$ 1.67 W/cm$^2$, 932.7 kHz in sine wave, duty cycle 20%, tone burst repetition frequency 10 Hz to 10 kHz) were adopted. Continuous wave induced a 50% transfection efficiency, and tone-burst ultrasound with a repetition frequency of 10 Hz led to 65% transferred cells [45].

Sonication has the potential of shearing/denaturing DNA through cavitation. It is therefore necessary to protect the DNA in order to maintain gene integrity. Investigators found that plasmid DNA is protected against cavitation induced damage when complexed with cationic liposomes [46].

Ultrasound can modulate gene expression in vitro and in vivo. Flow cytometry revealed that ultrasound

makes more transfected cells express target protein [45]. Similar findings were reported by Unger et al. [47]. Ultrasound with an intensity of 0.5 W/cm$^2$ and a frequency of 1 MHz enhanced gene expression in Hela, NIH/3T3 and C127I, into which DNA was introduced by liposomal transfection. Aggrecan gene expression was augmented by ultrasound exposure in a rat femur fracture model [48]. Artificial cavitation induced by contrast agents can increase gene transfection and its expression. This was confirmed in experiments performed by Bao et al. [49] and Greenleaf et al. [50]. These results suggest that cavitation is the main mechanism of ultrasound-induced transfer and expression. The transfection efficiency attributable to ultrasound was higher than that due to some other techniques. Encouragingly, ultrasound could transfer into quiescent cells with the same efficiency as that of proliferating cells.

Researchers believe that the ultrasound-induced transfection is mediated via a mechanism termed "sonoporation" [49], which was due to acoustic cavitation. Sonoporation can be considered to be the same as the promotion of membrane-permeability induced by ultrasound. Accordingly, only transient and repairable sonoporation can be applied to gene therapy. This indicates that ultrasound exposure should be administered precisely. The exact mechanisms of sonication-enhanced gene expression remains unclear, although investigators believe that non-thermal effects are the cause.

A temperature rise of 5–8 °C due to focused ultrasound exposure results in an expression of HSP mRNA in the focal region and the surrounding tissue with a index of 3–67 in rat muscle [51]. The promoter of HSP is sensitive to hyperthermia and this gives rise to a potential technique for controlling gene expression using ultrasound. Target DNA was inserted downstream of the HSP promoter, or other promoters that were sensitive to temperature. Such a DNA segment was introduced into target cells and then the gene expression could be modulated by altering the temperature in the tissue. Ultrasound could be used to induce the temperature rise quantitatively and precisely. This approach could regulate not only the expression within a specific region but also the expression level.

## 6. Apoptosis therapy

Apoptosis, (the normal sequence of events leading to cell death), is a frequent phenomenon in malignancies, however apoptosis therapy is an effective approach for cancer treatment. Apoptosis can occur spontaneously and be induced by many factors [52]. In cancer treatment it is induced either by radiotherapy or by chemotherapy. Malfunction of initiating apoptosis is one of the factors which result in the failure of such therapy [53]. Sonication can trigger apoptosis in both normal and malignant cells. Ultrasound-induced cell death has been confirmed in leukemia cell lines K562, HL-60, KG1a, Nalm-6 and U937 [54–57]. Contrast agents and dissolved gases enhance ultrasound-induced apoptosis but free radical scavengers can protect against this form
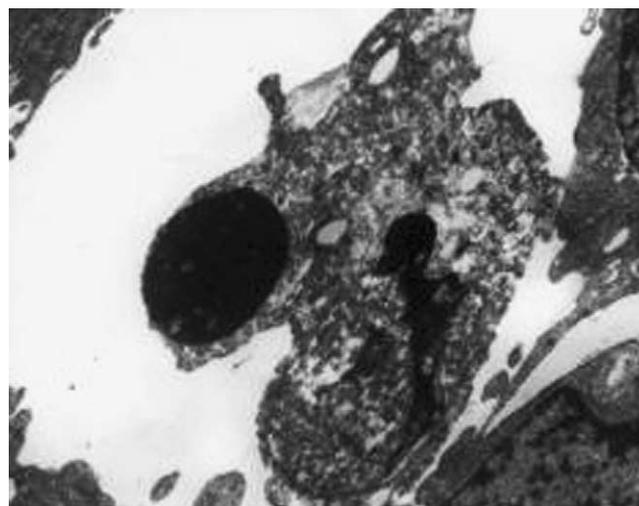


Fig. 4. Apoptotic body in human ovarian carcinoma cells after ultrasound exposure detected by transmission electron microscope.
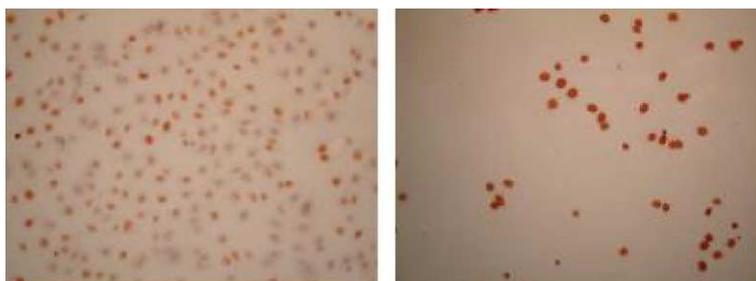


Fig. 5. Apoptosis in human ovarian carcinoma cell line detected by ISEL assay. Apoptotic cells are in brown and non-apoptotic cell in blue. Cells were exposed to adriamycin alone (left) and combination of adriamycin and sonication (right). Cell numbers decreased and apoptosis ratio increased when cells were treated with a combination of adriamycin and sonication.

of apoptosis [57]. Honda et al. suggest that ultrasound initiated apoptosis occurs via the mitochondria-caspase pathway [55]. Feril et al. report that the non-thermal effect of ultrasound (1 MHz, 0.5 W/cm$^2$) enhances the hyperthermia-induced (44 °C for 10 min) apoptosis in U937 cells but increasing the power to 1.0 W/cm$^2$ potentiates instant cell lysis [56].

We have investigated the effects of ultrasound on apoptosis in solid ovarian carcinoma. Flow cytometry revealed a sub-G1 peak after ultrasound exposure and this was confirmed by ultrastructural examination (Fig. 4). Furthermore, in situ end labelling (ISEL) showed that adriamycin-induced apoptosis was enhanced by ultrasound (Fig. 5). We have investigated this effect by evaluating the change of apoptosis ratio and that of cell survival with the elevation of adriamycin concentration. We believe that the lowering of the thresholds for both apoptosis and oncosis provide the mechanism for the synergism attributable to ultrasound exposure.

## 7. Other aspects

Ultrasound (1.5 W/cm$^2$, 20 kHz) can inhibit the adhesion and migration of smooth muscle cells [8]. On the other hand, neutrophil adhesion to endothelial cells was enhanced by therapeutic ultrasound (1.6 W/cm$^2$, 1.0 MHz) [58]. Investigators also found that low-level ultrasound could stimulate the synthesis and release of cytokines [9,59]. Potential values of these bioeffects for cancer therapy need further investigation.

## 8. Conclusions

Experimental investigations suggest that cancer therapy using low-level ultrasound is a promising technique and this type of ultrasound can also be co-administered with other therapeutic techniques. However, most findings indicate that the optimum frequency and power occurs over a narrow range. We believe that this has been one of the major restrictions to the effective use of low-level therapeutic ultrasound. As a result we conclude that given accurate dosage and careful administration the use of this methodology will become widespread.

One of the most important events which has contributed to the recent development of therapeutic ultrasound occurred in 2001 in Chonqing where a conference was held on the subject. During the conference a new society devoted to the promulgation of the general area of ultrasound in non-diagnostic medicine was established under the title "International Society for Therapeutic Ultrasound". Since then the society has organized other international meetings throughout the world.

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