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● *Review*

# A REVIEW OF LOW-INTENSITY ULTRASOUND FOR CANCER THERAPY

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**Abstract—The literature describing the use of low-intensity ultrasound in four major areas of cancer therapy—sonodynamic therapy, ultrasound-mediated chemotherapy, ultrasound-mediated gene delivery and anti-vascular ultrasound therapy—was reviewed. Each technique consistently resulted in the death of cancer cells, and the bio-effects of ultrasound were attributed primarily to thermal actions and inertial cavitation. In each therapeutic modality, theranostic contrast agents composed of microbubbles played a role in both therapy and vascular imaging. The development of these agents is important as it establishes a therapeutic–diagnostic platform that can monitor the success of anti-cancer therapy. Little attention, however, has been given either to the direct assessment of the mechanisms underlying the observed bio-effects or to the viability of these therapies in naturally occurring cancers in larger mammals; if such investigations provided encouraging data, there could be prompt application of a therapy technique in the treatment of cancer patients. (E-mail: Sehgalc@uphs.upenn.edu)** © **2015 World Federation for Ultrasound in Medicine & Biology.**

***Key Words:*** **Low-intensity ultrasound, Cancer therapy, Sonodynamic therapy, Ultrasound-mediated chemotherapy, Anti-vascular ultrasound, Ultrasound bio-effects, Microbubble contrast agent.**

## INTRODUCTION

Low-intensity ultrasound has been used in a variety of therapeutic applications. Together with sensitizing molecules it has been used to affect cancer cells (sonodynamic therapy); it has enhanced the activity of chemotherapeutic molecules in cancer therapy (ultrasound-mediated chemotherapy); it has been used to affect cells and their components directly (sonoporation); it has been used for gene delivery or transfection and to promote bone and tissue heating/healing and for its anti-vascular actions on tumor neovasculature. This appraisal of the literature focuses on the role of low-intensity ultrasound in cancer therapy. The published studies have included *in vitro* observations of cancer cell suspensions and cultures and the treatment of an extensive range of implanted tumors in small laboratory animals. This review covers four of the major areas in which low-intensity ultrasound has been used for cancer therapy studies: sonodynamic therapy, ultrasound-mediated chemotherapy, ultrasound-mediated gene delivery and anti-vascular ultrasound therapy.

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To date there is no widely accepted definition of low-intensity ultrasound, but this review has centered on investigations in which cancer cells or tumors have generally been insonated with an intensity less than 5.0 W $cm^{-2}$, corresponding to a root-mean-square pressure amplitude of about 0.3 MPa. Many variable sonication conditions have been used for the studies in the literature, making it difficult to make accurate comparisons between the reports. To aid the comparisons in this review, pressure–intensity conversions were made using the formula $I = p^2/\rho c$, where $I$ = intensity, $p$ = root mean square pressure amplitude, $\rho$ = density and $c$ = sound speed (Preston 1991).

In general terms, insonation of neoplasms with low-intensity ultrasound is easy to perform as it does not require a focused beam (that must be accurately located), the apparatus is relatively inexpensive, the bio-effects in adjacent normal tissues are commonly believed to be minimal and it is possible to easily target sensitizing or chemotherapeutic molecules and microbubbles located within the lumens of the tumor neovasculature. Treatment times are, however, prolonged in comparison to those used in high-intensity focused ultrasound, but repeated treatments or dose fractionation is easily performed.

## SONODYNAMIC THERAPY

The term *sonodynamic therapy* derives from photodynamic therapy. However, unlike photodynamic therapy, in which photosensitizers are excited directly by light to produce reactive oxygen species, sonodynamic therapy is mediated via ultrasound-induced cavitation and sonosensitizers to produce free radicals that kill nearby rapidly dividing cancer cells (Fig. 1). An attraction of sonodynamic therapy, in which continuous, low-intensity ultrasound at diagnostic ultrasound frequencies is used, is its ability to treat deeply located tumors. On the other hand, photodynamic therapy uses visible light, which attenuates rapidly in tissues, has limited penetration and can be employed only superficially or intraoperatively. When comparing the efficacy of the two methods, Jin et al. (2000) treated a subcutaneously located murine squamous cell carcinoma and found that sonodynamic therapy inhibited tumor growth by 77%, compared with 27% for photodynamic therapy. The latter was not as effective a therapy in the deeper regions of the tumor.

Sonodynamic therapy initially used the same light-sensitive agents, hematoporphyrin and its derivatives, that had been developed for photodynamic therapy. An ideal sensitizing agent should be preferentially taken up and retained in the tumor so that the therapy damages cancer cells, but has minimal bio-effects in the surrounding normal tissues; the agent should also be relatively non-toxic to normal mammalian tissues. To improve the efficacy of treating solid tumors, it is important that the sonosensitizer is injected intravenously before insonation, rather than directly into the tumor, so that it is more fully and evenly distributed throughout the neoplasm (Ninomiya et al. 2012).

Overviews of the sonosensitizers used in the therapy have been published (Chen et al. 2014; Feril et al. 2011; Kuroki et al. 2007; Shibaguchi et al. 2011). In sonodynamic therapy, the sonication parameters (usually 1.0–2.0 MHz at an intensity of 0.5 to 3.0 W $cm^{-2}$) (Tables 1 and 2) have been selected to produce inertial cavitation in a cell culture or tumor, where microbubbles rapidly collapse resulting in shockwaves that produce free radicals and a cascade of molecular events that activate the sonosensitizer and, in turn, damage the cancer cells (Misik and Riesz 2000; Rosenthal et al. 2004; Yu et al. 2004c). Although the production of reactive oxygen species appears important in the anti-tumor affect, Wang et al. (2011a) stated that thermal effects cannot be excluded. In addition to these direct cytotoxic effects on neoplastic cells, it is also important to consider other possible effects on the growing tumor, including its vascular supply. Gao et al. (2013) reported that sonodynamic therapy also has an anti-vascular effect and inhibits tumor neovascularization. Another approach has been to use a chemotherapeutic agent as the sonosensitizer. In *in vitro* studies of adriamycin (Gao et al. 2010), cisplatin (Bernard et al. 2011, 2012 [0.4 ± 0.02 MPa]) and doxorubicin (Liang et al. 2013), it was found that these agents were cytostatic, and apoptosis was further enhanced when they were used in combination with chlorine e6 (Gao et al. 2010) or a hematoporphyrin (Liang et al. 2013).

After the initial descriptions of sonodynamic therapy by Yumita et al. (1989) and Umemura et al. (1990), there were numerous confirming reports that further revealed the bio-effects of the therapy. In contrast to the earlier reviews, we have grouped the research studies according to the type of cancer cell and accompanying sonosensitizer that were insonated; the aim was to provide a guide to previous sonodynamic studies in which the type of cancer receiving therapy is emphasized (Tables 1 and 2). Considerable data have been published over the past 25 y using many different sonosensitizers and involving many types of cancer (Tables 1 and 2), and each report has consistently indicated the significant bio-effects of sonodynamic therapy. The relative merits of each of these numerous sonodynamic agents are, however, difficult to determine as each of the agents was investigated in isolation without comparing the efficacy of one against another. Thus, key questions remain to be answered, for example: Are the recently developed nanoparticle sonosensitizers any more effective than the original porphyrins in killing cancer cells?

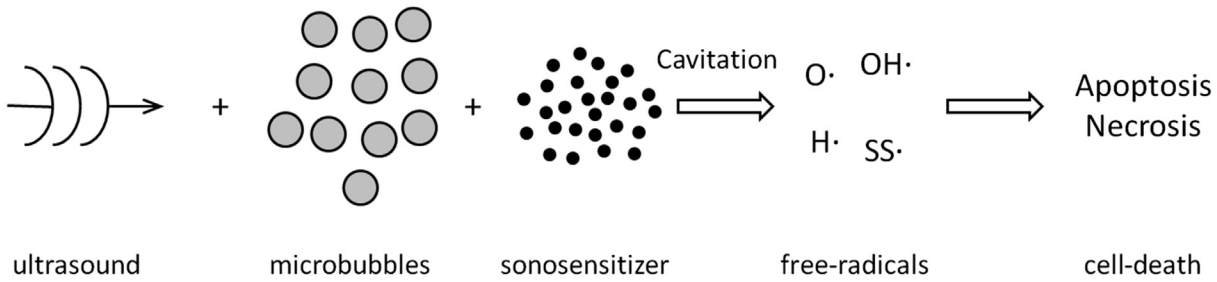


Fig. 1. Schema of sonodynamic therapy. Low-intensity insonation of cancer cells in the presence of a sonosensitizer causes cavitation, leading to the production of free radicals with resultant cell death by apoptosis and necrosis.