

Review

## Sonodynamic Therapy of Cancer Using Novel Sonosensitizers

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**Abstract.** Sonodynamic therapy (SDT) of cancer is based on preferential uptake and/or retention of a sonosensitizing drug (sonosensitizer) in tumor tissues and subsequent activation of the drug by ultrasound irradiation. Ultrasound can penetrate deeply into tissues and can be focused into a small region of a tumor to activate a sonosensitizer. This is a unique advantage in the non-invasive treatment of nonsuperficial tumors when compared to laser light used for photodynamic therapy. Recently, it has been found that photochemically active porphyrins also show significant antitumor effects when activated with ultrasound. The mechanism of sonodynamic action has been suggested to involve photoexcitation of the sensitizer by sonoluminescent light, with subsequent formation of singlet oxygen. This mini-review provides a brief overview of the following four sonosensitizers useful in SDT: i) a homogeneous complex of oligomers of hematoporphyrin, Photofrin II; ii) a gallium porphyrin complex, ATX-70; iii) a hydrophilic chlorin derivative, ATX-S10, and iv) a novel porphyrin derivative devoid of photosensitivity, DCPH-P-Na(I).

Photodynamic therapy (PDT) is a promising approach to the treatment of tumors in which photosensitizers, mainly hematoporphyrin or hematoporphyrin derivatives (HPDs), are accumulated in tumor tissues and excited by exposure to the appropriate wavelength of laser light, resulting in tumor cell killing by activated oxygen produced by the photosensitizers (1). PDT has been clinically applied to various types of cancer, including lung, colon and bladder

cancer (2-6). However, successful application of PDT is limited to superficial lesions of the tissues since penetration of laser light into tumor tissues is an important factor influencing the depth of PDT-induced cell damage. On the other hand, ultrasound has an appropriate tissue attenuation coefficient to penetrate intervening tissues and reach non-superficial objects while maintaining the ability to focus energy into small volumes (7). This is a unique advantage when compared to laser light in the application of non-invasive treatment of non-superficial tumors. In 1989, Yumita *et al.* found that several HPDs also induced significant cell damage when activated with ultrasound (8). It has since been demonstrated that several newly-generated HPDs have potential to be used as sonosensitizers for tumor treatment in combination with ultrasound (9-14), which is referred to as sonodynamic therapy (SDT) (7). This mini-review provides a brief overview of four novel sonosensitizers for SDT.

### Sonosensitizers Useful in SDT as well as in PDT

Photochemically active HPDs, including hematoporphyrin (8), Photofrin II (12, 13), ATX-70 (7, 9, 10, 14), and ATX-S10 (11), have been demonstrated to induce cell killing when activated by ultrasound irradiation, thus indicating that these chemicals originally generated for PDT are therefore applicable as sonosensitizers for tumor treatment in combination with ultrasound.

Photofrin II, a homogeneous complex of oligomers of hematoporphyrin (Figure 1), is retained preferentially in tumor tissues much longer than in normal tissues and has been approved by the FDA as a photosensitizer in PDT of cancer. Recently, Yumita *et al.* investigated the pharmacokinetics and tissue distribution of Photofrin II and its efficacy in sonodynamic therapy in rats bearing AH130 solid tumors (11) as well as in mice bearing colon 26 carcinoma (12). In order to find the optimal timing of the ultrasound exposure after injection of Photofrin II, its

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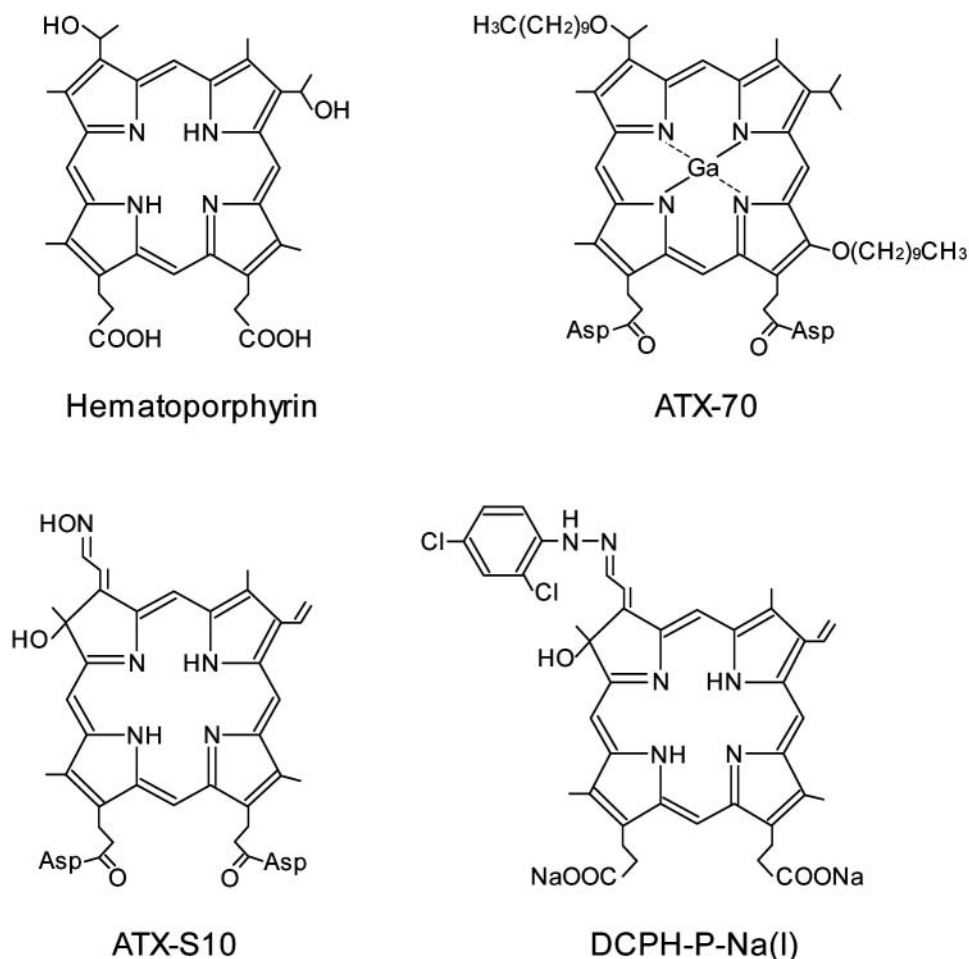


Figure 1. Chemical structures of hematoporphyrin, ATX-70, ATX-S10, and DCPH-P-Na(I). As shown in the text, Photofrin II is a homogeneous complex of oligomers of hematoporphyrin. The exact chemical names of the other three HPDs are as follows: 7,12-bis(1-decyloxyethyl)-2,18-bispropionylaspartic acid 3,8,13,17-tetramethyl-porphynate gallium(III) salt (ATX-70), 4-formylloximethylidene-3-hydroxy-2-vinyl-deuterio-porphynyl(IX)-6,7-diaspartic acid (ATX-S10), and 13,17-bis(1-carboxyethyl)-8-[2-(2,4-dichlorophenyl-hydrazono)ethylidene]-3-ethenyl-7-hydroxy-2,7,12,18-tetramethylchlorin, disodium salt [DCPH-P-Na(I)]. Asp denotes an aspartic acid residue of amino acid.

concentrations in plasma, muscle, skin and tumor were assayed and pharmacokinetically analyzed. Antitumor effects were estimated by measuring tumor size. Since the highest concentration of Photofrin II in tumors was observed 24 h after administration, ultrasound administration 24 h after the intravenous administration of Photofrin II was chosen. Ultrasound alone exhibited a slight antitumor effect, which became increasingly significant as the dose of Photofrin II was increased, while Photofrin II alone showed no significant effect. These results indicated that Photofrin II significantly sensitized solid tumors to the antitumor effect of ultrasound in a synergistic manner.

ATX-70, a gallium porphyrin complex (Figure 1), has shown the longest phosphorescence lifetime, much longer than those of other HPDs, and has been found to accumulate in tumors at the highest concentration (15).

Significant tumor tissue destruction has been demonstrated using ATX-70 in combination with pulsed laser light irradiation. Umemura *et al.* found that the rate of damage to isolated mouse sarcoma 180 cells in air-saturated suspension induced by ultrasound irradiation was enhanced more than four times by ATX-70, in contrast to only twice by the same concentration of hematoporphyrin (7). Maruyama *et al.* also reported that *in vitro* the cell damage to mouse MH134 hepatoma cells after ultrasound irradiation was enhanced by adding ATX-70 and that, *in vivo*, ultrasound irradiation and ATX-70 combination therapy inhibited cell growth (16). However, neither ultrasound irradiation alone nor ATX-70 treatment alone inhibited cell growth. These results imply that the antitumor effects of ultrasound irradiation and ATX-70 combined therapy are caused by activation of ATX-70 by ultrasound

irradiation. Furthermore, Yumita *et al.* evaluated the antitumor effect of ATX-70 induced by focused ultrasound, on colon 26 carcinoma cells implanted in a mouse kidney (9). ATX-70 was administered intravenously, 24 h before the ultrasonic exposure. The destruction of tumor tissue was observed with the ultrasonic treatment in combination with ATX-70, while neither the treatment with ATX-70 alone nor that with ultrasound alone caused any necrosis. These results demonstrated that the antitumor effects of ATX-70 could be induced by focused ultrasound. Similar antitumor effects of ATX-70 and ultrasound have been reported against mouse squamous cell carcinoma cells (10), human gastric adenocarcinoma cells (14) and human leukemia HL-525 cells (17).

ATX-S10, a chlorin derivative (Figure 1), has also shown a much longer phosphorescence lifetime than that of other HPDs and showed significantly lower toxicity than ATX-70 (15). The lethal dose of ATX-S10 to mice is an order of magnitude higher than that of ATX-70. Furthermore, ATX-S10 is also more preferentially retained by tumors than normal tissues. Nakajima *et al.* studied ATX-S10 accumulation in colon 26 tumor tissue after intravenous injection. The highest concentration of ATX-S10 in tumor tissue was observed 6 h after the administration and the ratio of concentration between the tumor and other normal tissues reached about five. Significant tumor tissue destruction was demonstrated using ATX-S10 in combination with pulsed laser irradiation. Recently, Yumita *et al.* investigated the sonodynamically induced antitumor effect of ATX-S10 (11). Both *in vitro* and *in vivo* antitumor effects were tested in combination with ultrasound. The rate of ultrasonically induced damage to isolated mouse sarcoma 180 cells in air-saturated suspension was enhanced two-fold with ATX-S10. The coadministration of ATX-S10 followed by ultrasonic exposure stopped the growth of implanted colon 26 tumors at an intensity at which ultrasound alone showed only a slight antitumor effect. Thus a significant ultrasonically induced antitumor effect as well as significant enhancement of ultrasonically induced *in vitro* cell damage has been demonstrated with ATX-S10.

### New Sonosensitizer Only Effective in SDT

As mentioned above, photochemically active HPDs including Photofrin II, ATX-70 and ATX-S10 have been demonstrated to induce cell killing when activated by ultrasound irradiation, thus indicating that these chemicals originally generated for PDT are therefore applicable as sonosensitizers for tumor treatment in combination with ultrasound. However, skin sensitivity to sunlight is still a major side-effect to be solved for photosensitizers. To improve the SDT of cancer using photosensitizers, we have

recently developed a novel porphyrin derivative designated as DCPH-P-Na(I) (Figure 1) and investigated its photochemical characteristics and sonotoxicity on tumor cells (18). DCPH-P-Na(I) exhibited minimum fluorescent emission by excitation with light in comparison to strong emission from ATX-70, which is known to reveal both photo- and sonotoxicity. According to this observation, when human tumor cells were exposed to light in the presence of DCPH-P-Na(I) *in vitro*, much less phototoxicity was observed in contrast to the strong phototoxicity of ATX-70. However, DCPH-P-Na(I) exhibited a potent sonotoxicity on tumor cells by irradiation with ultrasound *in vitro*. DCPH-P-Na(I) demonstrated significant sonotoxicity against a variety of cancer cell lines derived from different tissues. In addition, in a mouse xenograft model, potent growth inhibition of the tumor was observed by sonication after the administration of DCPH-P-Na(I) to the mouse. These results suggest that sonodynamic therapy with DCPH-P-Na(I) may therefore be a useful clinical treatment for carcinomas located deep in the human body without inducing skin sensitivity (18).

### Cell Killing Mechanism of Sonosensitizers

In PDT, when a photosensitizer is exposed to specific wavelengths of light, it is activated from its ground state into an excited state, and as the activated sensitizer returns to the ground state, the energy released can generate reactive oxygen species, such as singlet oxygen, and free radicals which mediate the direct cellular toxicity (19). The mechanism of the cytotoxicity in SDT seems to be theoretically similar to that in PDT. In SDT, it has been proposed that the activation of HPDs through acoustic cavitation by ultrasound is attributed to the generation of active oxygens (20, 21). Ultrasound irradiation induces cavitation around the surface of the tumor cell, which can produce white noise, sonochemical reactions, rupture of living cells and the emission of light, or sonoluminescent light. When a sonosensitizer attached to the surface of a tumor cell is exposed to the sonoluminescent light, it is activated from its ground state into an excited state, and as the activated sonosensitizer returns to the ground state, the energy released can generate reactive oxygen species, such as singlet oxygen, and free radicals which mediate cellular toxicity directly (Figure 2). In our study, L-histidine, a reactive oxygen scavenger of singlet oxygen and hydroxyl radicals (22, 23), reduced the sonotoxicity of DCPH-P-Na(I), but D-mannitol, a scavenger of hydroxyl radicals, hardly affected the sonotoxicity, thus suggesting that singlet oxygen is more important than hydroxyl radicals for the sonotoxicity of DCPH-P-Na(I) (18).

Cell killing by PDT and SDT has been shown to be the result of apoptosis and/or necrosis (24, 25). We were unable

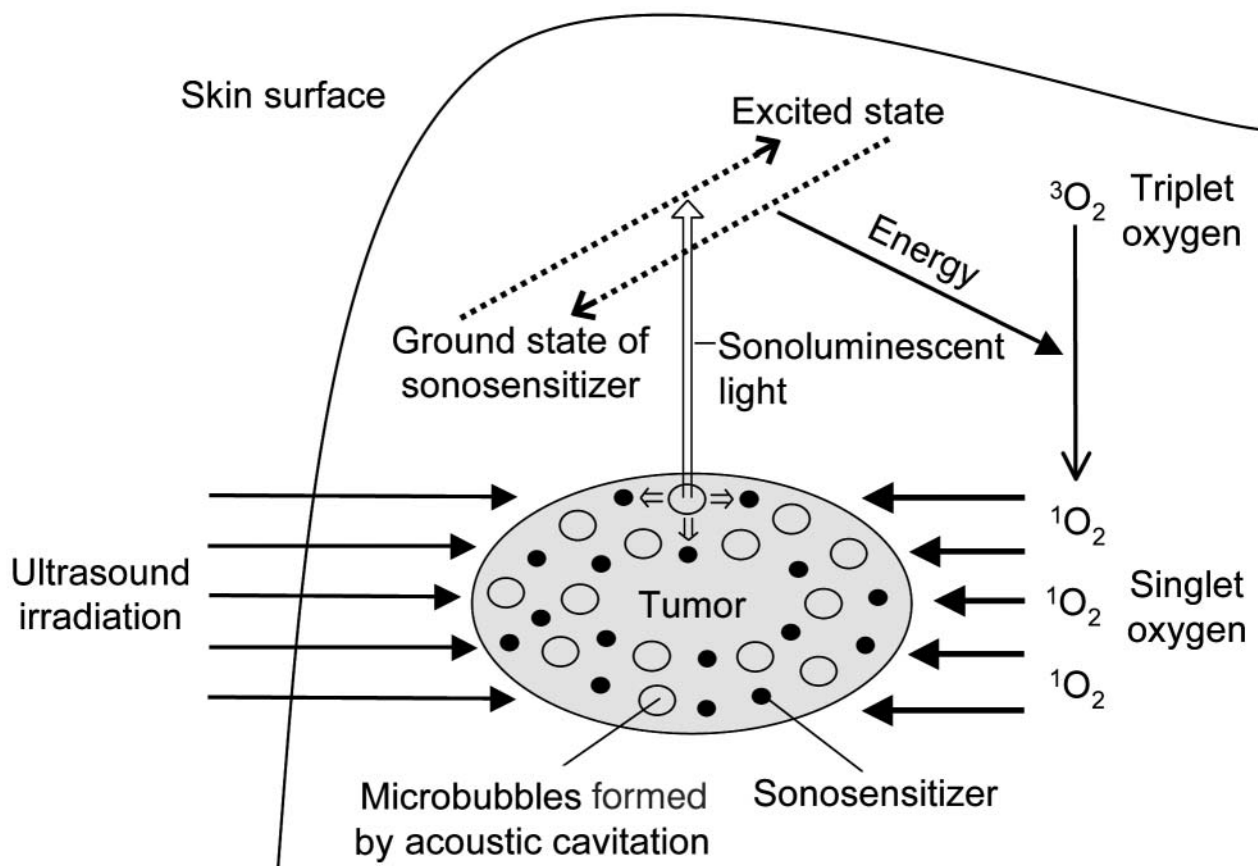


Figure 2. Possible mechanism of the cytotoxicity in SDT. Ultrasound irradiation induces cavitation around the surface of tumor cells, which can produce sonoluminescent light. A sonosensitizer, which tends to attach to the surface of tumor cells, is exposed to the sonoluminescent light and becomes activated from its ground state into an excited state. As the activated sonosensitizer returns to the ground state, the energy released can generate reactive oxygen species, such as singlet oxygen, which mediate the direct cellular toxicity.

to demonstrate that apoptosis is involved in the sonotoxicity of DCPH-P-Na(I). Previous studies have shown that the mode of cell death depends on different experimental conditions, including the cell type, the concentration of photosensitizers and incubation conditions (26-28). In addition to its direct phototoxicity to tumor cells, PDT affects the tumor vasculature so that the supply of oxygen and nutrients to tumor cells is hampered (29, 30), while also stimulating the immune systems by inducing inflammation at the irradiated site (31, 32). These secondary anti-tumor effects of PDT are also expected for SDT when using DCPH-P-Na(I).

### Conclusion

Ultrasound can penetrate deeply into tissues and can be focused into a small region of a tumor to activate a sonosensitizer. Hence, SDT using the novel sonosensitizers may be a useful tool for the treatment of cancer located too deep to be treated by regular PDT.

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