

Review

## Sono-Photodynamic Combination Therapy: A Review on Sensitizers

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**Abstract.** Cancer is characterized by the dysregulation of cell signaling pathways at several steps. The majority of current anticancer therapies involve the modulation of a single target. A tumor-targeting drug-delivery system consists of a tumor detection moiety and a cytotoxic material joined directly or through a suitable linker to form a conjugate. Photodynamic therapy has been used for more than 100 years to treat tumors. One of the present goals of photodynamic therapy research is to enhance the selective targeting of tumor cells in order to reduce the risk and extension of unwanted side-effects, caused by normal cell damage. Sonodynamic therapy is a promising new treatment for patients with cancer. It treats cancer with ultrasound and sonosensitive agents. Porphyrin compounds often serve as photosensitive and sonosensitive agents. The combination of these two methods makes cancer treatment more effective. The present review provides an overview of photodynamic therapy, sonodynamic therapy, sono-photodynamic therapy and the four sensitizers which are suitable candidates for combined sono-photodynamic therapy.

Cancer is a collection of over 100 diseases affecting all body organs. A single type of cancer presents itself differently in different individuals. The disease is a malfunction of the biochemical and signaling networks that drive the normal cell. The cell accumulates mutations and epigenetic changes gradually, which alter the signaling and biochemical

networks. Certain combinations of these alterations lead to cellular transformation and cancer (1). Signal transduction explains the conversion of external signals, generated by hormones, growth factors, neurotransmitters, chemokines, cytokines and even small molecules such as ATP, to a biochemical response, leading to a cellular response. These responses can lead to changes in metabolism, gene expression, cell division and cell death (2). The process can start at the cell membrane or inside the cell, leading to an array of biochemical reactions based on the signaling system (3). Recent reviews suggest that signal transduction therapy was implemented after the recognition of tyrosine phosphorylation as a key signaling event in incompetence in 1980 (4). Signal transduction therapy is generally thought of in relation to cancer therapy. This is because the most revolutionary developments in molecular, cellular biology are strongly related to cancer and carcinogenesis (5).

Phosphorylation is the most frequent post-translational modification of proteins and is intimately involved with several signal transduction processes (6).

Solid tumors are composed of two mutually-dependent compartments, the malignant cells and the tumor microenvironment, which includes the extracellular matrix, stromal cells and blood vessels (7). Inhibition of the stromal components that maintain the tumor is a valid strategy, if one can limit the lethal effects. One significant target is angiogenesis, *i.e.* the formation of new blood vessels (8).

There are four major types of cancer treatment: surgery, radiotherapy, chemotherapy, and immunotherapy. These therapies can be used either alone or in combination with each other (9). Many new treatments, including cancer vaccines and gene therapy, are being studied in clinical trials (10). Cancer chemotherapy has been one of the most important medical advances (11). Conventional cancer chemotherapy relies on the principle that rapidly proliferating cancer cells are more likely to be killed by cytotoxic agents. In contrast, targeted-therapy, introduced in recent years, is

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directed against cancer-specific molecules and signaling pathways and therefore has more limited non-specific toxicities (12). Tumor cells overexpress many receptors and biomarkers which can be used as targets to deliver cytotoxic agents into tumors (13). Tumor-targeting drug conjugates include monoclonal antibodies, polyunsaturated fatty acids, folic acid, hyaluronic acid, oligopeptides as tumor-targeting moieties, and tyrosine kinase inhibitors (14). In addition to targeted chemotherapy, another effective treatment of tumors is photodynamic therapy (PDT). PDT is a treatment that uses a drug, called a photosensitizer or photosensitizing agent, and a particular kind of light (15-17). When photosensitizers are exposed to a specific wavelength of light (18), they generate a form of oxygen that kills nearby cells (19). Sonodynamic therapy (SDT) has been developed as a complementary and alternative therapy to PDT. It treats cancer by means of ultrasound and sonosensitive agents (20).

## PDT

PDT has been used for more than 100 years to treat tumors. With suitable sensitizers, it has high efficacy, high selectivity, and few side-effects. There are good prospects for its broad clinical applications (21-23). PDT is carried-out in two steps, firstly by injecting or orally administering a photosensitive agent to a patient, and then activating the photosensitive agent by exposing the patient to light at an appropriate wavelength to produce singlet oxygen, thereby killing abnormal cells and microorganisms (24). Treatment with photosensitive compounds with an affinity for tumor cells allows for whole-body irradiation without damage to surrounding non-diseased cells. Photoporphyrins such as porphyrins, are molecules that produce active oxygen species after activation of the visible light wave range, and are widely used in PDT of cancer and other clinical conditions (25, 26). Chlorin e6 is a hydrophilic sensitizer derived from porphyrin (27). Chlorine e6 was found to have significantly higher photosensitizing activity of conjugates containing specific ligands, such as insulin, konkonavalin or folic acid, and thus is able to internalize receptor-expressing cells (28). Chlorine e6 has been shown to accumulate more effectively in tumors, absorb more strongly at longer wavelengths (670 nm) (29), be cleared faster from an organism, and can be activated by both light and ultrasound (30). PDT has the following advantages (31-35): (i) minimally-invasive nature, (ii) selectivity of the area of interest, and (iii) the ability to treat patients with repeated doses without initiating resistance or exceeding total dose limitations (as associated with radiotherapy).

## SDT

SDT has been developed as a complementary or alternative therapy to PDT. It treats cancer by means of ultrasound and

sonosensitive agents (30, 33-37). SDT is a procedure that uses ultrasound and a sonosensitizing compound that becomes cytotoxic upon exposure to ultrasound in order to treat cancer. It is similar to PDT in its advantage of low toxicity. The mechanism used is that ultrasound activates the sonosensitive agent in the body to generate singlet oxygen, thus killing abnormal cells such as tumor cells. Unlike PDT using light which has limited penetration, sound is able to penetrate deep into the body to reach interior tumors (20). Ultrasound is a mechanical wave with periodic vibrations of particles in a continuous, elastic medium at frequencies of 20 kHz or more. It is not only perceived as safe, but has exceptional tissue-penetrating ability without major attenuation of its energy (38, 39). The effect can be localized by focusing the ultrasound on a specific region and choosing compounds with tumor affinity (40-42).

## PDT and SDT: Comparison

Tumor- localizing porphyrin compounds were some of the first compounds used in the modern approach of SDT. Unlike chemotherapeutic compounds, porphyrins are non-cytotoxic in the absence of ultrasound. These compounds first found application in a method of treatment that is in some ways similar to SDT, PDT. PDT uses definite wavelengths of red light to activate the cytotoxic activity of photosensitive compounds. The application of PDT to tumor treatment is limited by the wavelength of red light, which only allows for limited tissue penetration (43). As a result, PDT can only treat superficial tumors that are at most 5 to 7 mm from the surface of the skin (44). Deeper tumors may be treated with interstitial irradiation; however, the minimal-invasive nature of PDT can be a hindrance to the procedure. However, this includes the typical non-invasive nature of PDT (45). SDT overcomes the shortcomings of PDT because the properties of ultrasound allow it to travel easily through many centimeters of tissue, allowing for the treatment of deeper tissues (46). Like high-intensity focused ultrasound (HIFU) treatment and other treatments utilizing the thermal effects of ultrasound, focused ultrasound is used in SDT to target a specific area and protect surrounding non-diseased tissues from activated sonosensitizers (47, 48). A targeted-approach works well for the treatment of solid tumors (49, 50). Porphyrin compounds have been used as photosensitive and sonosensitive agents (51). They selectively adhere to abnormal cells such as tumor cells.

## Sono-PDT

Sono-PDT is a safe, non-toxic and non-invasive way of destroying cancer cells, as well as of enhancing immune protective function. This treatment uses the light of a particular wavelength and sound of a particular frequency to

activate a light- and sonosensitive material which attaches selectively to tumor cells, causing their breakdown (52).

Both SDT and PDT have been used for years as separate processes and there are some 3000 published articles relating to their potential use in therapy (53). PDT is a licenced treatment in 19 countries and offered through the National Health Service in the United Kingdom. Both PDT and SDT have been used in the treatment of cancer, with variable success (54). PDT on its own is used for more superficial cancer types such as prostate, breast and skin cancer, but when combined with SDT, it has been shown to be efficient for deep-seated tumors such as bowel and ovarian cancer, as well as metastatic cancer, in particular when spread to bone, lung and liver tissues (55). The vast majority of patients with advanced cancer treated with sono-PDT live longer than predicted and in 75% of cases, there is significant tumor cell destruction (56). Sono-PDT is available as an effective treatment for cancer in England, Mexico, Israel, China and in Cape Town. At the beginning of the treatment, patients consume or are intravenously given a chlorophyll-based light-sensitive compound which binds selectively to tumor cells. It stays absorbed by cancer cells but is quickly released by healthy cells. The active agent is absorbed into the body 48-72 h prior to treatment and intravenous ozone is administered just before the treatment to enhance its effects; ozone, which is a super-oxygen compound, inhibits cancer cell growth, as cancer cells are more active under low oxygen thresholds. The patient is then placed in a specialized light bed and exposed to thousands of light emitting diodes (LED) which emit red and infrared light waves, followed by ultrasound treatment at a definite frequency in tumor-localized areas of the body. The excited sensitizer stimulates the formation of reactive oxygen species (ROS) from the molecular oxygen present in the cells. ROS lead the cancer cell to its death by severely increasing the level of oxidative stress, causing genetic and cell membrane damage. The death of cancer cells activates the immune system that responds to the call to clean-up the debris and attacks the remaining malignant cells that are finally recognized as invaders. Sono-PDT also blocks angiogenesis, the crucial conduit for cancer cell nutrition. Since the whole body is exposed to the light, all cancer or pre-cancerous cells within a range are affected, allowing the destruction and inhibition of cancer anywhere in the body (15, 57, 58). This treatment is entirely safe and the only side-effects are related to the destruction of the tumor cells that produce an inflammatory response intended at clearing the dead tumor tissue (59).

## Sensitizers

Sensitizers are the key factors for PDT and SDT. The synergistic effects of sensitizer and low-power ultrasound has been examined in many *in vitro* studies and to a lesser extent

in *in vivo* models (60). Sensitizer distribution and uptake in cells is potentially important for the therapeutic effect, because of the very short lifetime and very short diffusion distance of some radical products derived from the sensitizer produced during the procedure (61). Different sensitizers may have different mechanisms of action. Healthy cells have an aerobic mechanism. Cancer cells have an anaerobic metabolism and produce lactate. The sensitizer molecule with its positive charge binds to negatively-charged lactate in the cancer cell. It is less tightly held by healthy cells. Light or sound activation increases the energy level of the sensitizer, producing an activated molecule. This in turn reacts with nearby oxygen to form free radical oxygen. This is a super powerful oxidant, that it is relatively unstable and reacts with nearby oxidizable material, the organic matter in the cancer cell. This breaks down the organic matter, destroying the cell structure, and killing or damaging the cell. Free radical oxygen has a very small radius of action, so it only damages the cancer cells (62). At present, several experimental and clinical studies have provided convincing evidence that derivatives of chlorophyll a, in particular chlorin e6, are very promising compounds for PDT as far as they are characterized by high photodynamic activity and low frequency of known adverse reactions. One of the most promising chlorin e6-based photosensitizers officially approved for clinical use is Photolon® [also known as Fotolon® or chlorin e6-polymer polyvinylpyrrolidone (PVP) complex]. Photodynamic sensitizers have also been studied for ultrasound-activated properties. They have the benefit of being non-toxic unless activated and have been demonstrated to have a tumor-localizing properties (63, 64). Hematoporphyrin, a frequently used photo-sensitizer improved the killing of mouse sarcoma and rat ascites 130 tumor cells exposed *in vitro* to ultrasound (1.92 MHz) at intensities of 1.27 and 3.18 W/cm<sup>2</sup>, from 30% and 50% to 99% to 95%, respectively (65).

**Chlorine e6.** Chlorin e6 is a naturally-occurring chlorin derivative that has shown promise as a cancer therapeutic (66). Chlorin e6 is a second-generation photosensitizer with a high quantum yield of singlet oxygen of 0.65 at pH 7-8 (67). The chlorin e6 molecule has been adapted to improve its clinical efficacy, and has lesser side-effects compared to first-generation photosensitizers from hematoporphyrin derivatives (68). In addition, the amphiphilic structure of a chlorin e6 molecule allows for easy penetration through the cell membrane, thus ensuring accumulation inside cells (27, 69). Combination of ultrasound (1.6 W/cm<sup>2</sup>) with chlorin e6 (SDT) hampered tumor growth significantly ( $p < 0.05$ ) in SPCA-1 human lung adenocarcinoma cells and mice bearing SPCA-1 tumor xenograft. Flow cytometric analysis showed that chlorin e6-mediated sonodynamic effect occurred mainly through the induction of cell necrosis (70). Antitumor effect and possible mechanisms of chlorin e6-mediated sono-PDT

on murine 4T1 mammary cancer cells *in vitro* have suggested an enhanced therapeutic effect compared to each monotherapy. Caspase-dependent apoptosis, loss of matrix metalloproteinase, and generation of ROS are supposedly involved in the process (71).

**Photolon.** Photolon™/Fotolon™ is a water-soluble photosensitizer; the conjugation of chlorin e6 to hydrophilic PVP making Photolon hydrophilic in nature (72-75). While lipophilic photosensitizers (*e.g.* lipophilic porphyrins) are preferentially taken up through the plasma membrane of the cell, hydrophilic photosensitizers tend to be taken up by pinocytosis (76), a form of endocytosis, where particles are taken in by the cell in endosomal vesicles which subsequently fuse with lysosomes (*i.e.* lysosomotropic delivery) to exert its effects (75). Photolon addition to the cell suspension resulted in a significant enhancement of ultrasound cytotoxicity at 0.4 and 0.7 W/cm<sup>2</sup> intensity, with Light Intensity (LI<sub>50</sub>) decreasing to 0.33 W/cm<sup>2</sup>. The differences in cell number after exposure to ultrasound alone and ultrasound with Photolon were statistically significant ( $p < 0.01$ ). Thus, the gain in ultrasound cytotoxicity in the presence of Photolon was 1.5-fold (77). Combination treatment using photolon, including SDT and PDT, enhanced the effect on the glial tumor model in rat brain. The combination treatment significantly (by 25-30%) increased necrosis areas in tumor tissues compared with each of the components taken separately (78).

**Sonoflora 1.** Chlorophyll is a group of fat-soluble magnesium-porphyrin complexes widely found in green plants. It has good biomolecular affinity and is also a kind of broad-spectrum tumor inhibitor. As an efficient light-absorbing substance, its derivatives have been used as photosensitizers in PDT. This indicates that they may also be promising sonosensitizers. They are quickly absorbed by all cells upon oral administration. Within 24 hours, normal cells expel such agents, but they remain in cancer cells. When sound and light frequencies are pulsed through the body, they cause photosynthesis, which releases free radical oxygen directly into the cancer cells and kills them. Tumors shrink by up to 30% in the first 14 days of treatment (79). Sonoflora 1 is an analog of chlorophyll, in that its macrocycle backbone is porphyrin-based and the center of the porphyrin ring consists of a metal ion (80). The chlorophyll derivative has light absorption peaks at 402 and 636 nm (81). A very unusual combination of this investigational agent and various prototype light sources and ultrasound devices was used in the treatment of three patients who suffered from late-stage breast cancer with systemic metastases to multiple organs. All patients had significant partial or complete responses, thus proving sonopDT to be a promising new therapeutic combination for the treatment of breast cancer (82).

**Sonnelux-1.** Sonnelux-1 is a metallo-chlorin complex, containing a highly purified mixture of a number of chlorophyllins, each with a different side chain and an average molecular weight of 942. Sonnelux-1 has photo-activation properties and has also been demonstrated to be extremely sensitive to ultrasound (83). Sonnelux-1 has shown significant tumor cell cytotoxicity following ultrasound activation using a mouse S-180 sarcoma model (84). Tumor-growth inhibition was observed in the group that was administered both ultrasound and Sonnelux-1, with significant reduction in mean tumor weight. Activated cancer therapy using Sonnelux-1 shows prominence over a period of time. Activated cancer therapy using Sonnelux-1 was a well-tolerated non-invasive treatment even in advanced metastatic cancer and was a well-tolerated non-invasive treatment even in advanced metastatic cancer. No adverse events have been reported following administration of Sonnelux-1 (85).

### Light and Sound Sources for SPDT

The range of wavelengths of sound and light used in PDT and SDT are specific to the sensitizer—they need to be in the range that can cause the activation of the sensitizer. While light, by nature, is not penetrative; the water in the body can be used as a carrier to transmit sound deep into the body. As a result, the combination of light and sound allows us to target tumors at various depths in the body (86). Light source and light delivery are two of the primary aspects in PDT. The choice of light source for PDT can be determined by the location of the tumor, by the light dose applied and by the choice of photosensitizer (87). Lasers and lamps have both been employed to perform PDT and the advantage of one source over the other has not been demonstrated, hence the use of lasers or lamps depends on the particular application. Conventionally, red laser light with 630-nm wavelength has been proposed to activate Photofrin because of the increased tissue penetration of light at longer wavelengths. Red laser light is commonly produced by using an argon ion or KTP/YAG laser beam that is converted by a dye module to 630 nm. This conversion is inherently costly and not effective, but allows for light delivery with fiber optics. For non-fiber optic application of light, other light sources could potentially be useful alternatives (88). Although PDT has been traditionally performed using lasers, the availability of broad-band sources (lamps) is challenging the use of lasers where light can be delivered directly to the tumor (skin, oral cavity, *etc.*) without the need to combine the source with an optical fiber. A pulsed LED light source is both easy to use, as well as many times more penetrative than a regular light source of the same wavelength (89).

Ultrasound energy has been widely investigated and used over the past three decades in a broad range of clinical procedures (90). Sonication with high-intensity focused



ultrasound is an effective local cancer treatment that induces cytotoxicity through thermal effects, and non-thermal cavitation which generates intracellular ROS (62, 91, 92). Ultrasonically-induced cavitation is the major cause of sonoluminescence and sonochemical reactions (93) and, if it can be controlled, may have maximum potential for therapeutic applications among the non-thermal effects of ultrasound. The non-thermal nonolytic efficiency of a given low-level of ultrasound exposure has been correlated with the generation of acoustic cavitation (94, 95). Ultrasonically-induced cavitation, defined as generation and oscillation of gas bubbles, may cause permanent cell damage and modify the membrane structure and functional properties of the cells to induce cell killing by cell lysis, necrosis or apoptosis (96-98). SDT is an efficient local cancer treatment that induces cytotoxicity through thermal effects and non-thermal cavitation which generates intracellular ROS causing oxidative damage to a variety of cellular targets and subsequent tumor necrosis. Studies of the *in vivo* effects of ultrasound in animal brains have shown both thermal and cavitation mechanisms that depend on the applied intensity, ultrasound frequency and period of exposure. The effects of cavitation strongly depend on the tissue type and location. Furthermore, hemorrhage and blood vessel damage could occur when cavitation is present (99, 100). When high frequency sound waves are concentrated on body tissues, those tissues heat-up and die. To use this as a cancer treatment, the specialist targets the area containing the cancer. High-intensity focused ultrasound can be a successful treatment for prostate cancer. Recently, magnetic resonance imaging- or ultrasound-guided high-intensity focused ultrasound has been developed, not only for prostate cancer but also for liver cancer (101).

## Summary

Targeted cancer therapy is directed against cancer-specific molecules and signaling pathways and thus has more limited non-specific toxicities. PDT and SDT have been used in the treatment of cancer with variable success. Sono-PDT is a late-model and promising anticancer therapy using the combination of SDT and PDT. The efficacy of cell damage induced by chlorin e6-, Photolon-, Sonoflora-1- and Sonnelux-1-mediated combination therapy of SDT and PDT has rarely been reported. Developing advanced sensitizer materials which are suitable for SPDT therapy can improve the range of cancer treatment methodologies.

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