REVIEW ARTICLE

Sonodynamic Therapy in the Treatment of Glioblastoma: Mechanisms, Challenges, and Insights.

Abstract

Sonodynamic therapy is a rapidly evolving approach in cancer treatment, which uses ultrasound waves and sonosensitisers, drugs which are sensitive to ultrasound. Stemming from photodynamic therapy, it works by exposing sonosensitisers in tumour cells to high-intensity ultrasound, generating reactive oxygen species that cause cell damage. Among existing therapies, it is minimally invasive with fewer side effects and has the potential to enhance treatment efficacy when combined with other strategies. Glioblastoma multiforme has proven to be a hard-to-treat cancer with many existing treatments having limited efficacy against it due to the presence of the blood-brain barrier. This novel treatment is promising in the context of glioblastoma multiforme because it is less invasive than other treatments and potentially more effective in overcoming blood-brain barrier limitations. Nevertheless, every treatment has its drawbacks; Sonodynamic therapy relies on sonosensitisers, which are currently limited in their efficacy, and the lack of real-time monitoring of parameters in this treatment can lead to uncontrolled cytotoxic effects. This paper addresses the mechanisms of sonodynamic therapy, its application in combination with other therapies, its disadvantages and, more importantly, suggests solutions to its drawbacks.

Keywords

Translational Medical Sciences; Disease Treatment and Therapies; Sonodynamic Therapy; Ultrasound; Glioblastoma Multiforme

Introduction

To this day, cancer has become the second leading cause of global mortality, and anticancer treatment has attracted more and more researchers' attention.[1] Although current cancer treatment methods, such as chemotherapy, radiotherapy and immunotherapy, have proven to be reliable, they are not always curative. In treating glioblastoma multiforme (GBM), existing anticancer strategies have modest effectiveness in recurrent GBM, with the five-year rate of survival for patients being only 6.9%.[2–4] Sonodynamic therapy (SDT) is a novel cancer therapy treatment that works by activating pre-administered sonosensitive drugs with ultrasound (US) and generating reactive oxygen species (ROS), which are responsible for cell damage.

Chemotherapy and radiotherapy have poor tumour selectivity and can lead to increased therapy resistance through enhanced DNA repair mechanisms and hypoxia-inducible factor 1.[5] Therefore, the need for better tumour-targeted treatment may be resolved by SDT, which offers localised treatment and high selectivity to tumour cells, minimising damage to healthy tissue.[6] SDT was inspired by photodynamic therapy (PDT), a method of treatment using visible light to induce tumour necrosis. In 1989, several photosensitisers, which were hematoporphyrin derivatives (HPDs), were found to be sensitive to US and had the ability to induce cell damage when activated.[7] Compared to using visible light to induce tumour necrosis, US was shown to be more effective, as Jin et al. found that sonodynamic therapy inhibited tumour growth in murine squamous cell carcinoma in mice by 77%. In contrast, photodynamic therapy caused tumour growth inhibition of only 27%. Light waves cannot penetrate beyond a few millimetres of soft tissue, while high-intensity focused ultrasound (HIFU) has a penetration depth of up to 12 cm in soft tissue.[8,9]

Despite its potential advantages, SDT faces obstacles that must be overcome before it is fully recognised as an adjunctive treatment. Current sonosensitisers all have drawbacks, including poor bioavailability and selectivity, low reactive oxygen species (ROS) yield, and post-treatment side effects.[10] Another challenge associated with SDT is the lack of real-time monitoring of ROS and temperature. Too high temperatures may cause excessive production of ROS, leading to unwanted cell damage, whereas temperatures that are too low may not produce sufficient ROS needed to cause apoptosis. Therefore, it is crucial to monitor ROS concentration and temperatures during treatment.[11] The challenges associated with SDT will be covered in more detail in this paper, along with its potential solutions, which are accompanied by more research and in vivo studies.

Mechanisms in Sonodynamic Therapy

The primary way that SDT leads to cell death is the production of ROS when a sonosensitiser 'activates' as a result of microbubble cavitation, which occurs when sound waves at a certain frequency range permeate through aqueous environments.[12] To understand each step of this mechanism, we start with the cavitation effect caused by microbubbles. Microbubbles are microscopic air/oxygen bubbles ranging from 1-100 μm, which have great potential in site-specific drug delivery.[13] These microbubbles already exist in aqueous environments, but can be deliberately administered intravenously to patients to increase the efficacy of SDT.[14] Under US exposure, they undergo cyclic expansion and contraction in a process known as cavitation, which induces rapid temperature and pressure changes. Cavitation can either be 'stable' or 'inertial', where microbubbles experience violent, uncontrolled oscillations at higher acoustic pressures.[15] Inertial cavitation can subsequently cause sonosensitisers to rise to a higher energy state, leading to cytotoxicity through two possible mechanisms: sonoluminescence and pyrolysis. Sonoluminescence occurs when collapsing microbubbles release energy in the form of visible light.[16] Since most sonosensitisers are derived from photosensitisers, they are sensitive to light and will shift from their ground state to an excited state with higher energy. As they return to the ground state, they release energy to surrounding oxygen molecules to produce ROS, such as singlet oxygen, superoxide and hydrogen peroxide, which can damage intracellular DNA, promote lipid peroxidation, and result in apoptosis to targeted tumours, as shown in Figure 1.[17] Pyrolysis is similar in many ways; the only difference is that microbubbles shift sonosensitisers to an excited state by generating extreme amounts of thermal energy, rather than light. It is believed that inertial cavitation produces shockwave effects which thermally dissociate water vapour into hydroxyl radicals and hydrogen radicals even without the presence of a sonosensitiser.[18]

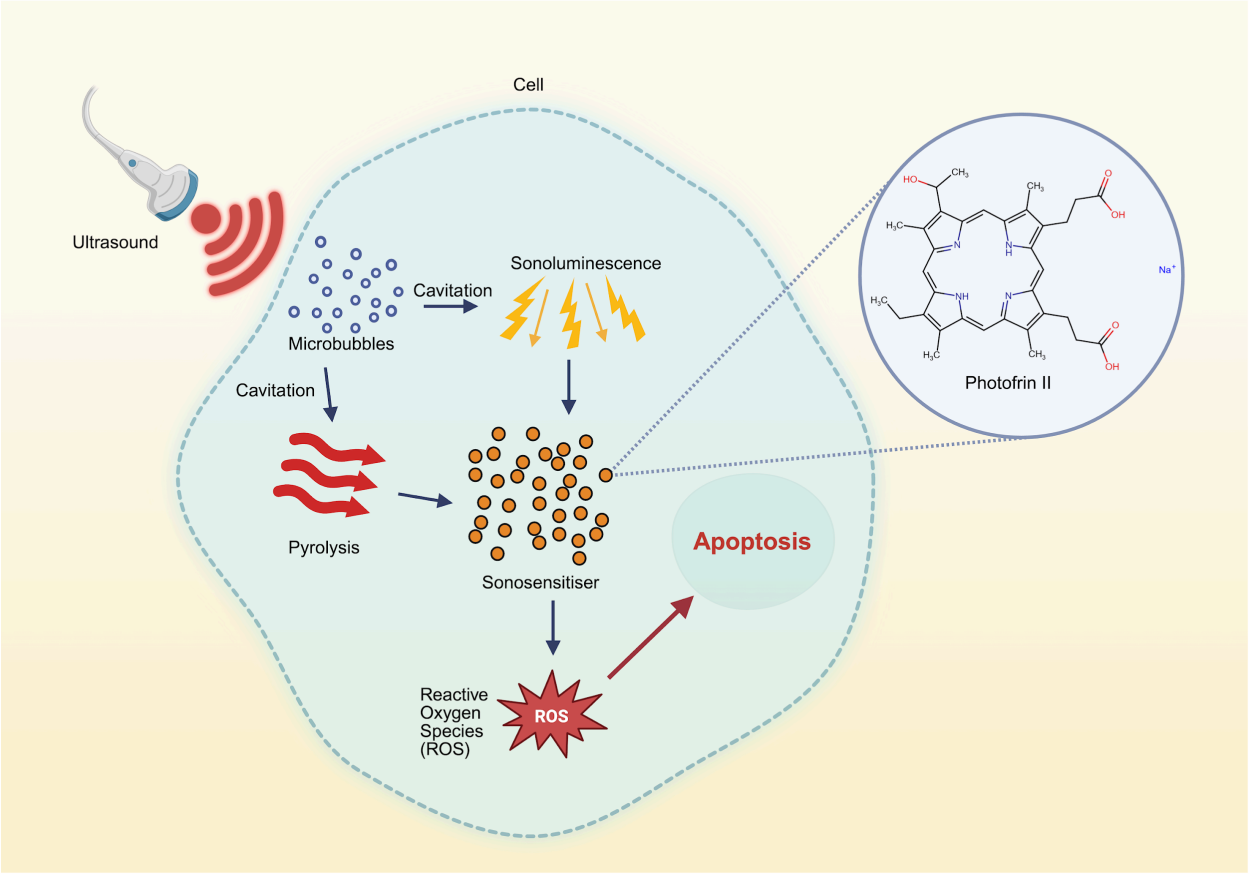

Figure 1: Schematic diagram to show microbubble cavitation leading to the generation of reactive oxygen species through sonoluminescence and pyrolysis, with Photofrin II as an example of a sonosensitiser. Figure created using https://BioRender.com and https://chemaxon.com/.

Microbubbles have a greater role than cavitation alone; they can enhance blood-brain barrier (BBB) permeability to aid drug entry into tumours and increase tumour specificity. This is especially useful in GBM, where the presence of the BBB prevents certain drugs from entering. In fact, the BBB blocks all large molecules and 98% of micro-molecule drugs from entering the tumour.[19] However, microbubble cavitation can cause significant shear stress on tumour cells and create ruptures in cell membranes in a process called cell lysis, thus enhancing the permeability of the tumour for sonosensitisers.[20,21] Another possible way microbubbles enhance permeability in tumour cells is through the generation of ROS, as mentioned earlier. Even without a sonosensitiser, microbubbles can generate ROS when in contact with US through pyrolysis, which is another possible explanation for increased tumour permeability.[22] Furthermore, microbubbles can improve targeted drug release. Glioma tumours are rich in vascular endothelial growth factor (VEGF), which is what promotes angiogenesis.[23] Currently, Carmustine (BCNU) is a widely used chemotherapeutic drug against GBM, despite its short half-life. However, Fan et al. designed VEGF-conjugated BCNU-loaded microbubbles which specifically bind to VEGF receptor two overexpressed in GBM tumours, thus allowing localised drug release at the tumour site and reducing damage to healthy tissue.[24] This indicates that microbubbles further increase the efficacy of SDT by targeted treatment and increased permeability in the tumour.

Organic Sonosensitisers

Sonosensitisers have a greater ability to selectively accumulate in tumour sites due to the enhanced permeability and retention (EPR) effect in tumours.[25] Ever since 1989, when Yumita et al. found that several hematoporphyrin derivatives (HPDs) used in PDT were sensitive to ultrasound, they have become the main focal point in sonosensitiser development, with Photofrin II and ATX-70 inducing severe cell damage when activated by US.[26,27] Despite having relatively good tumour specificity and ROS generation ability, even these commonly researched organic sonosensitisers have their downsides. Table 1 summarises the advantages and disadvantages of each sonosensitiser, both organic and inorganic, including their antitumour abilities, side effects and accumulation in tumours.

Photofrin II

Photofrin II is a family of oligomers where multiple hematoporphyrin molecules are bonded together, with the ability to retain in tumours longer than in healthy tissue selectively and has been FDA-approved as a photosensitiser in cancer PDT.[28] It was observed by Yumita et al. in 2000 that the highest concentration of Photofrin II in tumours was 24 hours post-administration, allowing time for the sonosensitiser to leave healthy tissue. In vivo studies in rats and mice with colon 26 carcinoma showed that Photofrin II had significant inhibition on tumour growth;[27] While ultrasound on its own had a mild antitumour effect, when targeted with Photofrin II, there was a clear antitumour effect. The results indicated that the antitumour effect strengthened increasingly as the Photofrin II dose increased.[28] A study on the effects of Photofrin II compared the survival rates of leukemic (MT-2) and normal peripheral mononuclear cells (PMNC). Using ultrasound (450 kHz, 0.3-0.5 W/cm^2), MT-2 cell survival decreased with higher sensitiser concentrations, while healthy cells showed no difference in survival rate between groups with and without Photofrin II administration. In patients with acute-type adult T cell leukaemia, PMNC survival decreased from 69.4% ± 22.5% with ultrasound alone to 30.0% ± 23.0% with Photofrin.[29] Although survival rates of leukemic cells are still relatively high, Photofrin II dropped leukemic cell survival rates by half. At the same time, healthy tissue showed no difference in survival rates, suggesting that Photofrin II has minimal toxicity to healthy cells. However, since porphyrins are photosensitive, they may cause post-administration side effects, including skin sensitivity to light and external burns.[30] Furthermore, their low water solubilities call for the use of nanocarriers to enhance accumulation in tumour tissues.

ATX-70

ATX-70 is a gallium porphyrin complex which showed the highest accumulation concentration in tumours out of the HPDs.[31] It also exhibits the longest phosphorescence lifetime among HPDs. In other words, ATX-70 absorbs and reemits sonoluminescent light the longest while also selectively accumulating in tumours. The efficacy of ATX-70 was studied using isolated sarcoma 180 cells, and findings demonstrated that an 80 μM concentration of ATX-70 increased the rate of damage to the mice sarcoma by four times under 2 MHz US, compared to the two times increased rate with hematoporphyrin.[32] These findings suggest that ATX-70 significantly amplifies US-induced cytotoxic effects, primarily through the generation of singlet oxygen radicals, and potentially has a greater effect than Photofrin II and other HPDs. The in vivo effects of ATX-70 in Sprague-Dawley rats with mammary tumours were examined and showed that peak accumulation of ATX-70 in tumours was 24 hours post-administration, much like Photofrin II.[33] Furthermore, ATX-70 concentration in the tumour was significantly higher than in plasma, muscle and skin cells. 2 weeks after administration of ATX-70 and US radiation (3 W/cm^2), there was a mean reduction of tumour volume of 50%, and with radiation of 5 W/cm^2, a further 18% reduction in tumour volume was observed compared to the control group (no treatment). This study concluded that ATX-70 significantly enhanced tumour regression when coupled with US and confirmed its efficacy as a sonosensitiser. Other studies reported similar observations on mouse squamous cell carcinoma cells and colon adenocarcinoma models.[34,35] Other HPDs, such as ATX-S10, have also shown antitumour effects and preferential retention in tumours.[36] Nevertheless, ATX-70 is a porphyrin derivative and shares the same downsides as Photofrin II.

Rose Bengal

Another organic compound, Rose Bengal (RB), a xanthene dye, has also been tested for its potential cytotoxic effects when exposed to US. RB increased ultrasound-induced cell damage to isolated sarcoma 180 cells by a factor of 2-3 at a concentration of 160 μM.[37] At a US exposure of 5.9 W/cm², cell viability dropped to as low as 4% in the presence of 160 μM RB, but only to 38% with US alone after 60 seconds. However, comparing the concentrations from their previous studies, they observed that to achieve similar cytotoxic effects, RB concentration needed to be double or more than double the concentration of porphyrins, suggesting that porphyrins have a slight advantage in efficacy in terms of cell damage.[12,26,32] Xanthene dyes are also more limited in that the liver can easily capture them and are unable to accumulate in tumours.[38]

Inorganic Sonosensitisers

Titanium dioxide (TiO_2) nanoparticles

Inorganic sonosensitisers have also been researched. Titanium dioxide (TiO_2) nanoparticles (NPs) are a class of metal-based sonosensitisers that also produce ROS when activated by ultrasound.[39] As a superconducting material, it can produce electron-hole pairs when excited by ultrasound.[40] These pairs interact with nearby oxygen and water molecules to produce hydroxyl radicals and superoxide anions. An in vitro study suggested that TiO_2 NPs or US alone had little effect on mouse melanoma C32 cells, with cell viability remaining above 92% after US exposure at intensities up to 1.0 W/cm^2 for 10 seconds. However, combining a TiO_2 solution (0.500% w/w) with US at 1.0 W/cm² for 10 seconds resulted in a decrease in cell viability to 53.6 ± 1.8%, and apoptotic cell levels were 2.73 times higher than those in the control group. Additionally, microscopic analysis confirmed that the TiO_2 particles were responsible for cell membrane damage, indicating the production of reactive oxygen species (ROS).[41] Despite its potential apoptosis-inducing ability, TiO_2 NPs tend to aggregate in physiological environments, which enables them to be easily captured by the reticuloendothelial system.[39] Therefore, research on carrier systems that can improve the dispersion stability of TiO_2 NPs under physiological pH conditions will significantly enhance the efficacy of TiO_2 NPs as sonosensitisers in SDT. You et al. encapsulated TiO_2 NPs in carboxymethyl dextran (CMD). This long-circulating hydrophilic TiO_2 NP still retained the ability to produce ROS when activated by US, but improved systemic circulation and uptake into cells.[42] Another study used an encapsulation of TiO_2NPs in polyion complex micelles to improve their dispersion stability while producing singlet oxygen.[43] TiO_2 NPs possess another drawback, namely, electron-hole recombination: As mentioned earlier, TiO_2 produces electrons and holes upon exposure to ultrasound. However, electrons and holes can recombine quite easily, resulting in reduced ROS production.[40] A strategy that involves doping noble metals, such as gold or platinum, into TiO_2 NPs helps improve the rate of ROS production. Doping gold, for example, into TiO_2 boosts the generation of ROS such as hydroxyl radicals and singlet oxygen; Noble metals act as electron traps, extending electron lifetimes and enhancing ROS production by reducing electron-hole recombination, as shown by Perota et al. when they developed Au/TiO_2 nanocomposite for the combination treatment of Phototherapy and SDT of melanoma in vitro.[44] Other metal-based sonosensitisers that include Manganese-based composites and Fe3O4-loaded sensitisers also show antitumour effects, but may be limited in the complexity of their design and their reduced ROS production rate under non-acidic and hypoxic conditions, meaning that strategies to overcome these are important.[39]

Silicon nanostructures

Non-metal sonosensitisers have also been widely used in SDT research. Silicon nanostructures, for instance, can cause amplified sonodynamic effect, making them a potential sonosensitiser. A study found that silicon nanoplatforms (SiNPs) had significant cytotoxic effect when paired with US: While US (0.88 MHz, 0.05 W/cm^2) alone only led to 50% Hep-2 cancer cell death in vitro, when combined with SiNPs (0.2 mg/ml), 90% of the cancer cells were destroyed.[45] The researchers also conducted in vivo experiments on

mice with Lewis lung carcinoma (LLC). The mice received injections of a SiNP suspension (1 mg/ml) into the tumour. When exposed to US, tumour growth was inhibited by 30 ± 5% over the course of 13 days, further suggesting that SiNP may be a possible sonosensitiser in SDT. Furthermore, Mesoporous silica nanoparticles (MSNs) have been modified in the past to alter their hydrophilicity, enabling microbubbles to be trapped in a hydrophobic MSN and increasing ROS production.[39] However, despite showing some promise in vitro and in vivo, the efficacy and safety of SiNP in human clinical trials remain unclear.

Type	Sonosensitiser	Advantages	Disadvantages	Reference
Organic	Photofrin II	• FDA-approved for PDT • Significant tumour growth inhibition in vivo • Good accumulation in tumours	• High phototoxicity • Low water solubility	[27–30]
Organic	ATX-70	• Significant tumour growth inhibition in vivo • Good accumulation in tumours	• High phototoxicity • Low water solubility	[31–33,35]
Organic	Rose Bengal (RB)	• Good tumour growth inhibition	• Requires higher concentration than porphyrins • Easily captured by the liver • Poor accumulation in tumours	[12,32,37,38]
Inorganic	Titanium dioxide nanoparticles (TiO_2 NPs)	• Good tumour growth inhibition • Can be modified to improve circulation and ROS production	• Aggregates and can be captures by the reticuloendothelial system • Electron-hole recombination reduces ROS production	[39–44]
Inorganic	Silicon nanoparticles (SiNPs)	• Medium tumour growth inhibition • Hydrophobic SiNPs can carry microbubbles to increase ROS production	• Antitumour effects are not as strong as those of porphyrins	[39,45]

Table 1: Presents the advantages and disadvantages of inorganic and organic sonosensitisers.

Discussion

Sonodynamic Therapy and Glioblastoma

GBM is classified as a World Health Organisation (WHO) grade IV astrocytoma, meaning it is highly malignant. The standard treatment for glioblastoma consists of surgery followed by radiotherapy and/or chemotherapy.[46] However, glioblastomas grow rapidly and are fatal, with a median survival time of 12.1 months with standard radiotherapy alone and 14.6 months when treated with both radiotherapy and temozolomide.[46]

In vitro experiments have demonstrated the potential of SDT in combating GBM. 5-Aminolevulinic acid (5-ALA), a precursor of porphyrins, has been extensively researched for its application in treating GBM. 5-ALA, at concentrations of 10 μm/mL, coupled with US at a power of 6 W, significantly reduced cell viability in rat RG2 glioma cells in vitro.[47] Similar observations were found in mouse cells in vitro as well as human U87 glioblastoma cells.[48,49] Other sonosensitisers, including rose Bengal, sinoporphyrin sodium and PpIX have also shown apoptotic effects in glioma cells.[50–52] Though SDT may not be a likely primary treatment in the future, it has high potential to become a supporting treatment. To date, research has shown that SDT can be combined with other therapies such as chemotherapy and PDT to yield synergistic effects.

Chemotherapy, as mentioned earlier, is a mainstream therapy used in GBM treatment. In the case of brain tumours, SDT can aid chemotherapy in a few ways. A major challenge associated with brain tumours is the presence of the BBB, which restricts molecules from entering the tumour site.[53] As mentioned earlier, cavitation of microbubbles can facilitate the entry of sonosensitisers into tumours.[54] By improving the selective uptake of chemotherapeutic drugs in cancer cells, SDT thereby reduces toxicity in normal cells and tissues. SDT also enhances the sensitivity of cancer cells to chemotherapeutic drugs by inducing apoptosis and inhibiting ATP-binding cassette (ABC) transporters, including ABCG2. These transporters actively efflux chemotherapeutics, which would normally lead to tumour resistance to chemotherapy. However, SDT disrupts mitochondrial function and activates the mitochondria-caspase apoptotic pathway, both of which contribute to reduced ABC transporter expression.[55] Hence, the role of US and sonosensitisers can theoretically be used alongside chemotherapy to enhance treatment efficacy. When Wang et al. investigated the potential synergistic cytotoxic effects of protoporphyrin IX (PpIX)-mediated SDT combined with chemotherapeutic drug doxorubicin (DOX), their findings demonstrated that SDT significantly enhanced the efficacy of DOX by inducing apoptosis and increasing intracellular drug uptake.[56] When SDT was coupled with DOX, the ABC transporter P-glycoprotein (P-gp) was inhibited by 21.3%, leading to an increased uptake of the drug in K562/DOX cells, a multi-drug-resistant human leukaemia cell line. While DOX alone caused apoptosis rates of 7.9%, and US and PpIX caused apoptosis rates of 13.9%, Wang et al. found that together, SDT and chemotherapy caused the highest apoptosis rate of 39.6%, suggesting the synergistic effects of SDT and chemotherapy.

SDT stems from PDT, and along with it, sonophotodynamic therapy (SPDT) emerged with the combination of SDT and PDT. PDT, however, relies on light, which, by nature, is not penetrative, while SDT relies on US waves, which can be passed through the body through water molecules. Therefore, activating sonosensitisers with a combination of US and light allows targeting tumours at different depths.[57] By activating sensitisers using both light and ultrasound simultaneously, SDPT exploits the synergistic effects of the two therapies to produce mechanical, sonochemical and photochemical activities, leading to apoptosis. Liu et al. conducted both in vitro and in vivo studies on the benefits of SPDT in human breast cancer cells and mouse mammary cancer cell lines, and found that SPDT was more effective than SDT or PDT alone.[58] In particular, while SDT alone caused a loss of cell viability of 27.36%-34.88% and PDT resulted in a loss of 36.69%-40.16% in vitro, the SPDT group exhibited a loss

of cell viability ranging from 80.49% to 85.01%. Further analysis revealed a 12.68% survival rate for SPDT-treated cancer cells, which is substantially lower than that of SDT and PDT (68.78% and 59.51%, respectively). The evident synergistic effects of SPDT can be attributed to the increased generation of ROS. Specifically, the SPDT group production of intracellular reactive oxygen species in the mice was 4-5 times more than SDT or PDT alone, suggesting that the heightened levels of ROS caused the apoptosis in SPDT. SPDT's effects on squamous cell carcinoma (SCC) tumour models were investigated.[59] While they found that PDT alone resulted in a 40% reduction in tumour volume and PDT combined with TiO_2 sensitisers achieved 55% growth inhibition, the SPDT group exhibited an 80% tumour suppression. Increased ROS generation caused this increased effectiveness; however, more electron spin resonance (ESR) measurements showed that specifically hydroxyl radicals were most prevalent in SPDT tumours, being three times higher than in SDT or PDT alone. The results of these studies indicate a synergistic effect when SDT is combined with PDT. Though PDT and SDT alone may have mild effects, when combined together, SPDT has the ability to treat deep-lying tumours that light would not reach, such as bowel and ovarian cancer or metastatic cancer that spreads to bone, lung and liver tissue.[60] SPDT is still a newly emerging combination, and its effects have still not been optimised in GBM treatment. A study found that in C6 rat models, its effects were initially promising, but its long-term impacts were less effective than PDT alone.[61] SDT surprisingly contributed to tumour growth, and a possible reason for this was that the US was incorrectly applied. Although the effects of SPDT were small, ROS production in the SPDT group was far greater than in the SDT or PDT group. Further research into sustaining SPDT's effects will be crucial for putting it into use in clinical settings.

Current Limitations and Insights of Sonodynamic Therapy

Sonosensitisers are a critical component of SDT, yet their development is still in progress. Due to a range of issues, including low stability, poor bioavailability, and selectivity, the current generation of sonosensitisers requires further research before SDT can become a more widely used treatment.[11] Some organic sonosensitisers, such as RB, for instance, have the ability to be triggered by US but are limited in their tumour accumulation ability. The lack of accumulation ability in some sensitisers has inspired research into the synthesis of their derivatives and investigations of their properties. For example, Sugita et al. investigated the development of tumour-accumulating derivatives of RB for SDT and PDT to overcome RB's poor tumour accumulation.[62] Several RB derivatives (RBD) were tested, including RBD1 (2, RB, C-2' alkyl ester, C-6 sodium salt), RBD2 (3, RB, C-2′ ω-carboxyalkyl ester, C-6 molecular form) and RBD3 (4, RB, C-2′ R-carboxyalkyl ester, C-6 molecular form). While all three derivatives behaved similarly to other sonosensitisers, RBD3 with longer alkyl chains significantly improved tumour accumulation, accumulating 1.5 times more in tumours than ATX-70 and 40 times more than RB. The researchers successfully engineered tumour-targeting RBDs by optimising amphiphilicity and showed that these modifications make RBDs promising sonosensitisers for SDT. To further combat the low selectivity of sonosensitisers, Xiong et al. isolated a novel porphyrin derivative from Photofrin known as sinoporphyrin sodium (DVDMS), which showed strong potential in SDT due to its water solubility.[63] They found that DVDMS concentrations in tumours in vivo reached approximately 98.77% and 70.37% of the maximum concentration in the tumour at 6 h and 24 h, respectively. In S180 xenografted mice, DVDMS-mediated SDT also significantly decreased tumour growth rates; It outperforms other porphyrin-based sonosensitisers due to its superior tumour selectivity, effective ROS generation and strong tumour inhibition, deeming it a promising solution, among others, to the drawbacks of current sonosensitisers.

The role of microbubbles can also be expanded in SDT. As mentioned earlier, microbubbles facilitate the activation of sonosensitisers through cavitation and pyrolysis. A promising potential of microbubbles was investigated by Nimokou et al., when they covalently attached RB to a lipid microbubble to form a conjugate, which showed heightened cytotoxicity in tumour cells and a greater reduction in tumour growth compared to RB alone.[64] First, microbubble conjugation provides site-specific, precise drug delivery, as

US selectively collapses microbubbles, activating only sonosensitisers around the tumour site. Second, the close spatial proximity of RB to the microbubble enhances the effectiveness of ROS production. In fact, when the cytotoxic potential of the MB-RB conjugate was assessed, it was found that exposure to US (1 MHz, 1.5 W/cm^2 for 30 s) decreased cell viability by 72% in RIF-1 murine fibrosarcoma cells in vitro. The results also indicated that the MB-RB conjugate had a significantly enhanced production of singlet oxygen compared to unconjugated RB, supporting the potential of MB-RB conjugates as a highly effective approach in SDT. Furthermore, microbubbles can be used to identify tumour locations more accurately: Zheng et al. developed hematoporphyrin-encapsulated poly(lactic-co-glycolic acid) microbubbles, which acted as a contrast agent for ultrasound imaging both in vitro and in vivo.[65]

Another possible function of microbubbles in SDT is to combat hypoxia levels usually associated with tumours. A compromised and anisotropic blood supply in tumours leads to inadequate oxygen and nutrient delivery to tumour cells.[66] As a result, SDT, which relies on oxygen acting as a substrate for the generation of ROS, becomes less effective and limited in its cytotoxic potential. To combat hypoxic environments, oxygen-loaded MBs (OxyMBs) conjugated with RB (OxyMB-RB) were investigated.[14] A series of in vitro and in vivo experiments was conducted. However, notable findings in the in vivo experiments revealed that OxyMB-RBs resulted in a 45% reduction in tumour volume over 5 days with US (1 MHz, 3.5 W/cm^2, 30% duty cycle, 3.5-minute exposure). In contrast, a non-oxygen-loaded MB conjugate (SF6MB-RB) resulted in a 35% increase in tumour volume. The significant difference in tumour growth between OxyMB-RB + ultrasound and SF6MB-RB + ultrasound indicates that the oxygen delivery is crucial for SDT efficacy in hypoxic tumours.

In both clinical applications and future studies, providing real-time monitoring of treatment parameters, such as ROS concentration, is crucial. Extreme concentrations of ROS generated may lead to temperatures too high, and can result in damage to surrounding healthy tissue; therefore, more research on optimising and standardising SDT parameters is beneficial.[11] A proposed insight into quantitatively measuring ROS concentration is through chemiluminescence, where probes detect the emission of light from chemical reactions occurring in SDT. Several researchers have developed chemiluminescence probes: Hu et al. developed a near-infrared area charge-coupled device which detected chemiluminescence in 2-dimensional imaging in vivo, which showed a linear relationship between singlet oxygen concentration and chemiluminescence, making quantification of ROS concentration possible in vivo.[67] Zhen et al. engineered chemiluminescent semiconducting polymer nanoparticles which were sensitive to chemiluminescent irradiation by hydrogen peroxide, and they were able to detect concentrations as low as 5 nM in vivo mouse models.[68]

Conclusion

SDT has been developed to treat various cancers, including GBM, through the generation of ROS, causing apoptosis. Numerous different sonosensitisers have been researched and tested. Currently, Photofrin II, an organic sonosensitiser, is being used in clinical applications for PDT. While it shows good antitumour effects, it is limited in its low water solubility and side effects on the patient. Inorganic sonosensitisers, however, show weaker antitumour effects but can be more easily modified to increase their efficacy. SDT has a high potential in treating GBM due to its ability to open up the BBB for a higher accumulation in tumours, especially when combined as an adjunctive treatment to other therapies, such as chemotherapy or PDT. However, more research and in vivo experimentation are needed to optimise this newly emerging therapy; it is limited by current sonosensitiser development and a lack of real-time monitoring of parameters, such as ROS concentration. Recent studies have revealed that microbubbles may have a greater role in optimising SDT since they can be conjugated to sonosensitisers to improve tumour accumulation or combat hypoxia conditions, which decrease the efficacy of SDT. Furthermore, utilising chemiluminescent probes in real time may improve the effectiveness of SDT in clinical applications, as well as enabling future studies to quantify ROS concentrations. SDT, however, will only be used in clinical applications through more experimentation and collaboration among researchers.

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Decision: Accept with moderate revisions

This is, for the most part, a well written paper on an interesting, original, and significant topic. The student has clearly engaged with the literature and most of the grammar is excellent. I only have a few points of feedback to provide. Here is the first: it is important to remember that the introduction is for readers who don't have a background in the field, and therefore anything you mention should come with some context or explanation as if the person reading your paper has never heard of these things before. For instance, you discuss cancer without mentioning its causes, the tumor microenvironment, lack of successful targeted therapies or immunotherapies for GBM, or the direct risks of radiation therapy. You also say that SDT is highly selective to tumour cells but don't provide an explanation - why is that the case?

My second point of feedback would be to shorten or break up some of the longer paragraphs, such as the first paragraph in the "Mechanisms in Sonodynamic Therapy" section and the "Photofrin II" and "ATX-70" and "Titanium dioxide nanoparticles" paragraphs. The third and fourth paragraphs in the "Sonodynamic Therapy and Glioblastoma" section are also quite long. Long chunks of text will cause your reader's eyes to become blurry and disengaged and they will skim over them. Adding figures or breaking them up into more digestible chunks will keep your reader engaged. My third suggestion is to discuss the risks of these treatments more. Are there any risks of microbubbles rupturing the blood brain barrier? Could this increase metastasis or cause other damage? Are there risks of getting metal sonosensitisers? How long do they stay in the body and how are they filtered out?

Lastly, the organization feels a bit strange. I think the "Discussion" title should go where the "Conclusion" title is currently, as having three sections in a discussion - two of which are still presenting data - is unusual. If these changes are made, I think this paper is a great candidate for publication.

My recommendation for the review paper named “Sonodynamic Therapy in the Treatment of Glioblastoma: Mechanisms, Challenges, and Insights.” is that it should be revised and resubmitted.

Overall, the topic of the paper is very interesting and discusses a relatively new area of cancer research – a fact that the student also acknowledged in their manuscript. This could also have been tricky, given the fact that resources available to the student would have been limited due to that. The student succeeded in finding enough resources. This was evident especially in the discussion section, where they argued about the advantages and disadvantages of the use of SDT in cancer treatment, providing solid insights to the reader. Moreover, the fact that many of the resources used are from the last 5 years show that the student engaged with recent literature.

The student also discussed future directions for this area of research, which shows a clear understanding of the topic, whilst including an element of a paper that is necessary.

The major reason for recommending this paper for revision lies especially in the introduction section. As I have included in the comments in the PDF, in the introduction, the student needs to talk about glioblastoma, current treatments, their effectiveness, and give a foundational reason about why the use of SDT in glioblastoma is important. Without this, it seems like the paper suddenly moves into a deeper insight without having all of the foundational ideas being presented in advance.

Adding to the above comment, the student generally needs to pay attention when they introduce new concepts which are vital to the paper. One example being GBM which I have touched upon before, other examples being the organic and inorganic sonosensitisers, where they should explain what these are before discussing them and their use in treatment.

Additionally, at the end of the section “Mechanisms in sonodynamic therapy”, I recommended that the student adds a few sentences that link that section to the next one, so that the paper can flow nicely and the reader will know what to expect afterwards. The same is true for the paragraph on ATX-70, where I felt that it ended a bit abruptly.

Therefore, by having to do work on the points above, the student would have to do a bit more research and include new references in the paper. This may seem as time consuming, but it will definitely help the student, both for this paper and for any future writing they will carry out.

There have been some instances where the student needs to rephrase some sentences for clarity, include references at the end of some other ones, fix some issues with abbreviations, or check the formatting of the paragraphs and pages. However, these are, to my judgement, minor revision points, that the student will definitely improve on with more experience in writing and reading academic papers.

To reiterate, the student has done a very good job on this paper and the feedback should not discourage them. After all, revision is a standard and normal practice of research writing. On the contrary, they should be proud of their achievement – creating their own piece of research – and use this feedback to work on the points I raised. In doing so, it would result in a paper that fits the modern scientific writing standards and would give them a solid foundation in academic writing, for the rest of their career.

REVIEW ARTICLE

Sonodynamic Therapy in the Treatment of Glioblastoma: Mechanisms, Challenges, and Insights.

Abstract

Sonodynamic therapy is a rapidly evolving approach in cancer treatment, which uses ultrasound waves and sonosensitisers, drugs which are sensitive to ultrasound. Stemming from photodynamic therapy, it works by exposing sonosensitisers in tumour cells to high-intensity ultrasound, generating reactive oxygen species that cause cell damage. Among existing therapies, it is minimally invasive with fewer side effects and has the potential to enhance treatment efficacy when combined with other strategies. Glioblastoma multiforme has proven to be a hard-to-treat cancer with many existing treatments having limited efficacy against it due to the presence of the blood-brain barrier. This novel treatment is promising in the context of glioblastoma multiforme because it is less invasive than other treatments and potentially more effective in overcoming blood-brain barrier limitations. Nevertheless, every treatment has its drawbacks; Sonodynamic therapy relies on sonosensitisers, which are currently limited in their efficacy, and the lack of real-time monitoring of parameters in this treatment can lead to uncontrolled cytotoxic effects. This paper addresses the mechanisms of sonodynamic therapy, its application in combination with other therapies, its disadvantages and, more importantly, suggests solutions to its drawbacks.

Keywords

Translational Medical Sciences; Disease Treatment and Therapies; Sonodynamic Therapy; Ultrasound; Glioblastoma Multiforme

Introduction

To this day, cancer has become the second leading cause of global mortality, and anticancer treatment has attracted more and more researchers' attention.[1] Although current cancer treatment methods, such as chemotherapy, radiotherapy and immunotherapy, have proven to be reliable, they are not always curative. In treating glioblastoma multiforme (GBM), existing anticancer strategies have modest effectiveness in recurrent GBM, with the five-year rate of survival for patients being only 6.9%.[2–4] Sonodynamic therapy (SDT) is a novel cancer therapy treatment that works by activating pre-administered sonosensitive drugs with ultrasound (US) and generating reactive oxygen species (ROS), which are responsible for cell damage.

Chemotherapy and radiotherapy have poor tumour selectivity and can lead to increased therapy resistance through enhanced DNA repair mechanisms and hypoxia-inducible factor 1.[5] Therefore, the need for better tumour-targeted treatment may be resolved by SDT, which offers localised treatment and high selectivity to tumour cells, minimising damage to healthy tissue.[6] SDT was inspired by photodynamic therapy (PDT), a method of treatment using visible light to induce tumour necrosis. In 1989, several photosensitisers, which were hematoporphyrin derivatives (HPDs), were found to be sensitive to US and had the ability to induce cell damage when activated.[7] Compared to using visible light to induce tumour necrosis, US was shown to be more effective, as Jin et al. found that sonodynamic therapy inhibited tumour growth in murine squamous cell carcinoma in mice by 77%. In contrast, photodynamic therapy caused tumour growth inhibition of only 27%. Light waves cannot penetrate beyond a few millimetres of soft tissue, while high-intensity focused ultrasound (HIFU) has a penetration depth of up to 12 cm in soft tissue.[8,9]

Despite its potential advantages, SDT faces obstacles that must be overcome before it is fully recognised as an adjunctive treatment. Current sonosensitisers all have drawbacks, including poor bioavailability and selectivity, low reactive oxygen species (ROS) yield, and post-treatment side effects.[10] Another challenge associated with SDT is the lack of real-time monitoring of ROS and temperature. Too high temperatures may cause excessive production of ROS, leading to unwanted cell damage, whereas temperatures that are too low may not produce sufficient ROS needed to cause apoptosis. Therefore, it is crucial to monitor ROS concentration and temperatures during treatment.[11] The challenges associated with SDT will be covered in more detail in this paper, along with its potential solutions, which are accompanied by more research and in vivo studies.

Mechanisms in Sonodynamic Therapy

The primary way that SDT leads to cell death is the production of ROS when a sonosensitiser 'activates' as a result of microbubble cavitation, which occurs when sound waves at a certain frequency range permeate through aqueous environments.[12] To understand each step of this mechanism, we start with the cavitation effect caused by microbubbles. Microbubbles are microscopic air/oxygen bubbles ranging from 1-100 μm, which have great potential in site-specific drug delivery.[13] These microbubbles already exist in aqueous environments, but can be deliberately administered intravenously to patients to increase the efficacy of SDT.[14] Under US exposure, they undergo cyclic expansion and contraction in a process known as cavitation, which induces rapid temperature and pressure changes. Cavitation can either be 'stable' or 'inertial', where microbubbles experience violent, uncontrolled oscillations at higher acoustic pressures.[15] Inertial cavitation can subsequently cause sonosensitisers to rise to a higher energy state, leading to cytotoxicity through two possible mechanisms: sonoluminescence and pyrolysis. Sonoluminescence occurs when collapsing microbubbles release energy in the form of visible light.[16] Since most sonosensitisers are derived from photosensitisers, they are sensitive to light and will shift from their ground state to an excited state with higher energy. As they return to the ground state, they release energy to surrounding oxygen molecules to produce ROS, such as singlet oxygen, superoxide and hydrogen peroxide, which can damage intracellular DNA, promote lipid peroxidation, and result in apoptosis to targeted tumours, as shown in Figure 1.[17] Pyrolysis is similar in many ways; the only difference is that microbubbles shift sonosensitisers to an excited state by generating extreme amounts of thermal energy, rather than light. It is believed that inertial cavitation produces shockwave effects which thermally dissociate water vapour into hydroxyl radicals and hydrogen radicals even without the presence of a sonosensitiser.[18]

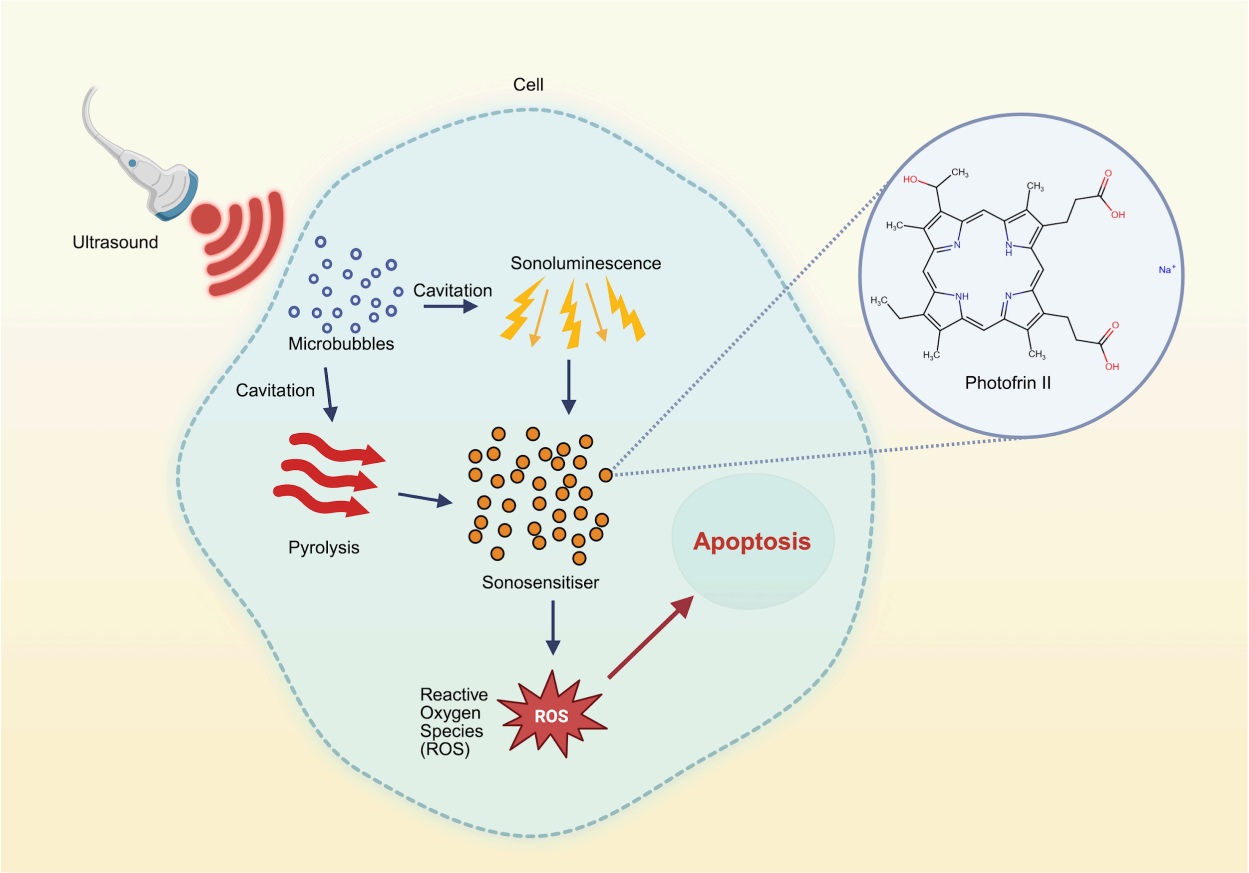

Figure 1: Schematic diagram to show microbubble cavitation leading to the generation of reactive oxygen species through sonoluminescence and pyrolysis, with Photofrin II as an example of a sonosensitiser. Figure created using https://BioRender.com and https://chemaxon.com/.

Microbubbles have a greater role than cavitation alone; they can enhance blood-brain barrier (BBB) permeability to aid drug entry into tumours and increase tumour specificity. This is especially useful in GBM, where the presence of the BBB prevents certain drugs from entering. In fact, the BBB blocks all large molecules and 98% of micro-molecule drugs from entering the tumour.[19] However, microbubble cavitation can cause significant shear stress on tumour cells and create ruptures in cell membranes in a process called cell lysis, thus enhancing the permeability of the tumour for sonosensitisers.[20,21] Another possible way microbubbles enhance permeability in tumour cells is through the generation of ROS, as mentioned earlier. Even without a sonosensitiser, microbubbles can generate ROS when in contact with US through pyrolysis, which is another possible explanation for increased tumour permeability.[22] Furthermore, microbubbles can improve targeted drug release. Glioma tumours are rich in vascular endothelial growth factor (VEGF), which is what promotes angiogenesis.[23] Currently, Carmustine (BCNU) is a widely used chemotherapeutic drug against GBM, despite its short half-life. However, Fan et al. designed VEGF-conjugated BCNU-loaded microbubbles which specifically bind to VEGF receptor two overexpressed in GBM tumours, thus allowing localised drug release at the tumour site and reducing damage to healthy tissue.[24] This indicates that microbubbles further increase the efficacy of SDT by targeted treatment and increased permeability in the tumour.

Organic Sonosensitisers

Sonosensitisers have a greater ability to selectively accumulate in tumour sites due to the enhanced permeability and retention (EPR) effect in tumours.[25] Ever since 1989, when Yumita et al. found that several hematoporphyrin derivatives (HPDs) used in PDT were sensitive to ultrasound, they have become the main focal point in sonosensitiser development, with Photofrin II and ATX-70 inducing severe cell damage when activated by US.[26,27] Despite having relatively good tumour specificity and ROS generation ability, even these commonly researched organic sonosensitisers have their downsides. Table 1 summarises the advantages and disadvantages of each sonosensitiser, both organic and inorganic, including their antitumour abilities, side effects and accumulation in tumours.

Photofrin II

Photofrin II is a family of oligomers where multiple hematoporphyrin molecules are bonded together, with the ability to retain in tumours longer than in healthy tissue selectively and has been FDA-approved as a photosensitiser in cancer PDT.[28] It was observed by Yumita et al. in 2000 that the highest concentration of Photofrin II in tumours was 24 hours post-administration, allowing time for the sonosensitiser to leave healthy tissue. In vivo studies in rats and mice with colon 26 carcinoma showed that Photofrin II had significant inhibition on tumour growth;[27] While ultrasound on its own had a mild antitumour effect, when targeted with Photofrin II, there was a clear antitumour effect. The results indicated that the antitumour effect strengthened increasingly as the Photofrin II dose increased.[28] A study on the effects of Photofrin II compared the survival rates of leukemic (MT-2) and normal peripheral mononuclear cells (PMNC). Using ultrasound (450 kHz, 0.3-0.5 W/cm^2), MT-2 cell survival decreased with higher sensitiser concentrations, while healthy cells showed no difference in survival rate between groups with and without Photofrin II administration. In patients with acute-type adult T cell leukaemia, PMNC survival decreased from 69.4% ± 22.5% with ultrasound alone to 30.0% ± 23.0% with Photofrin.[29] Although survival rates of leukemic cells are still relatively high, Photofrin II dropped leukemic cell survival rates by half. At the same time, healthy tissue showed no difference in survival rates, suggesting that Photofrin II has minimal toxicity to healthy cells. However, since porphyrins are photosensitive, they may cause post-administration side effects, including skin sensitivity to light and external burns.[30] Furthermore, their low water solubilities call for the use of nanocarriers to enhance accumulation in tumour tissues.

ATX-70

ATX-70 is a gallium porphyrin complex which showed the highest accumulation concentration in tumours out of the HPDs.[31] It also exhibits the longest phosphorescence lifetime among HPDs. In other words, ATX-70 absorbs and reemits sonoluminescent light the longest while also selectively accumulating in tumours. The efficacy of ATX-70 was studied using isolated sarcoma 180 cells, and findings demonstrated that an 80 μM concentration of ATX-70 increased the rate of damage to the mice sarcoma by four times under 2 MHz US, compared to the two times increased rate with hematoporphyrin.[32] These findings suggest that ATX-70 significantly amplifies US-induced cytotoxic effects, primarily through the generation of singlet oxygen radicals, and potentially has a greater effect than Photofrin II and other HPDs. The in vivo effects of ATX-70 in Sprague-Dawley rats with mammary tumours were examined and showed that peak accumulation of ATX-70 in tumours was 24 hours post-administration, much like Photofrin II.[33] Furthermore, ATX-70 concentration in the tumour was significantly higher than in plasma, muscle and skin cells. 2 weeks after administration of ATX-70 and US radiation (3 W/cm^2), there was a mean reduction of tumour volume of 50%, and with radiation of 5 W/cm^2, a further 18% reduction in tumour volume was observed compared to the control group (no treatment). This study concluded that ATX-70 significantly enhanced tumour regression when coupled with US and confirmed its efficacy as a sonosensitiser. Other studies reported similar observations on mouse squamous cell carcinoma cells and colon adenocarcinoma models.[34,35] Other HPDs, such as ATX-S10, have also shown antitumour effects and preferential retention in tumours.[36] Nevertheless, ATX-70 is a porphyrin derivative and shares the same downsides as Photofrin II.

Rose Bengal

Another organic compound, Rose Bengal (RB), a xanthene dye, has also been tested for its potential cytotoxic effects when exposed to US. RB increased ultrasound-induced cell damage to isolated sarcoma 180 cells by a factor of 2-3 at a concentration of 160 μM.[37] At a US exposure of 5.9 W/cm², cell viability dropped to as low as 4% in the presence of 160 μM RB, but only to 38% with US alone after 60 seconds. However, comparing the concentrations from their previous studies, they observed that to achieve similar cytotoxic effects, RB concentration needed to be double or more than double the concentration of porphyrins, suggesting that porphyrins have a slight advantage in efficacy in terms of cell damage.[12,26,32] Xanthene dyes are also more limited in that the liver can easily capture them and are unable to accumulate in tumours.[38]

Inorganic Sonosensitisers

Titanium dioxide (TiO_2) nanoparticles

Inorganic sonosensitisers have also been researched. Titanium dioxide (TiO_2) nanoparticles (NPs) are a class of metal-based sonosensitisers that also produce ROS when activated by ultrasound.[39] As a superconducting material, it can produce electron-hole pairs when excited by ultrasound.[40] These pairs interact with nearby oxygen and water molecules to produce hydroxyl radicals and superoxide anions. An in vitro study suggested that TiO_2 NPs or US alone had little effect on mouse melanoma C32 cells, with cell viability remaining above 92% after US exposure at intensities up to 1.0 W/cm^2 for 10 seconds. However, combining a TiO_2 solution (0.500% w/w) with US at 1.0 W/cm² for 10 seconds resulted in a decrease in cell viability to 53.6 ± 1.8%, and apoptotic cell levels were 2.73 times higher than those in the control group. Additionally, microscopic analysis confirmed that the TiO_2 particles were responsible for cell membrane damage, indicating the production of reactive oxygen species (ROS).[41] Despite its potential apoptosis-inducing ability, TiO_2 NPs tend to aggregate in physiological environments, which enables them to be easily captured by the reticuloendothelial system.[39] Therefore, research on carrier systems that can improve the dispersion stability of TiO_2 NPs under physiological pH conditions will significantly enhance the efficacy of TiO_2 NPs as sonosensitisers in SDT. You et al. encapsulated TiO_2 NPs in carboxymethyl dextran (CMD). This long-circulating hydrophilic TiO_2 NP still retained the ability to produce ROS when activated by US, but improved systemic circulation and uptake into cells.[42] Another study used an encapsulation of TiO_2NPs in polyion complex micelles to improve their dispersion stability while producing singlet oxygen.[43] TiO_2 NPs possess another drawback, namely, electron-hole recombination: As mentioned earlier, TiO_2 produces electrons and holes upon exposure to ultrasound. However, electrons and holes can recombine quite easily, resulting in reduced ROS production.[40] A strategy that involves doping noble metals, such as gold or platinum, into TiO_2 NPs helps improve the rate of ROS production. Doping gold, for example, into TiO_2 boosts the generation of ROS such as hydroxyl radicals and singlet oxygen; Noble metals act as electron traps, extending electron lifetimes and enhancing ROS production by reducing electron-hole recombination, as shown by Perota et al. when they developed Au/TiO_2 nanocomposite for the combination treatment of Phototherapy and SDT of melanoma in vitro.[44] Other metal-based sonosensitisers that include Manganese-based composites and Fe3O4-loaded sensitisers also show antitumour effects, but may be limited in the complexity of their design and their reduced ROS production rate under non-acidic and hypoxic conditions, meaning that strategies to overcome these are important.[39]

Silicon nanostructures

Non-metal sonosensitisers have also been widely used in SDT research. Silicon nanostructures, for instance, can cause amplified sonodynamic effect, making them a potential sonosensitiser. A study found that silicon nanoplatforms (SiNPs) had significant cytotoxic effect when paired with US: While US (0.88 MHz, 0.05 W/cm^2) alone only led to 50% Hep-2 cancer cell death in vitro, when combined with SiNPs (0.2 mg/ml), 90% of the cancer cells were destroyed.[45] The researchers also conducted in vivo experiments on

mice with Lewis lung carcinoma (LLC). The mice received injections of a SiNP suspension (1 mg/ml) into the tumour. When exposed to US, tumour growth was inhibited by 30 ± 5% over the course of 13 days, further suggesting that SiNP may be a possible sonosensitiser in SDT. Furthermore, Mesoporous silica nanoparticles (MSNs) have been modified in the past to alter their hydrophilicity, enabling microbubbles to be trapped in a hydrophobic MSN and increasing ROS production.[39] However, despite showing some promise in vitro and in vivo, the efficacy and safety of SiNP in human clinical trials remain unclear.

Type	Sonosensitiser	Advantages	Disadvantages	Reference
Organic	Photofrin II	• FDA-approved for PDT • Significant tumour growth inhibition in vivo • Good accumulation in tumours	• High phototoxicity • Low water solubility	27–30
Organic	ATX-70	• Significant tumour growth inhibition in vivo • Good accumulation in tumours	• High phototoxicity • Low water solubility	31–33,35
Organic	Rose Bengal (RB)	• Good tumour growth inhibition	• Requires higher concentration than porphyrins • Easily captured by the liver • Poor accumulation in tumours	12,32,37,38
Inorganic	Titanium dioxide nanoparticles (TiO_2 NPs)	• Good tumour growth inhibition • Can be modified to improve circulation and ROS production	• Aggregates and can be captures by the reticuloendothelial system • Electron-hole recombination reduces ROS production	39–44
Inorganic	Silicon nanoparticles (SiNPs)	• Medium tumour growth inhibition • Hydrophobic SiNPs can carry microbubbles to increase ROS production	• Antitumour effects are not as strong as those of porphyrins	39,45

Table 1: Presents the advantages and disadvantages of inorganic and organic sonosensitisers.

Discussion

Sonodynamic Therapy and Glioblastoma

GBM is classified as a World Health Organisation (WHO) grade IV astrocytoma, meaning it is highly malignant. The standard treatment for glioblastoma consists of surgery followed by radiotherapy and/or chemotherapy.[46] However, glioblastomas grow rapidly and are fatal, with a median survival time of 12.1 months with standard radiotherapy alone and 14.6 months when treated with both radiotherapy and temozolomide.[46]

In vitro experiments have demonstrated the potential of SDT in combating GBM. 5-Aminolevulinic acid (5-ALA), a precursor of porphyrins, has been extensively researched for its application in treating GBM. 5-ALA, at concentrations of 10 μm/mL, coupled with US at a power of 6 W, significantly reduced cell viability in rat RG2 glioma cells in vitro.[47] Similar observations were found in mouse cells in vitro as well as human U87 glioblastoma cells.[48,49] Other sonosensitisers, including rose Bengal, sinoporphyrin sodium and PpIX have also shown apoptotic effects in glioma cells.[50–52] Though SDT may not be a likely primary treatment in the future, it has high potential to become a supporting treatment. To date, research has shown that SDT can be combined with other therapies such as chemotherapy and PDT to yield synergistic effects.

Chemotherapy, as mentioned earlier, is a mainstream therapy used in GBM treatment. In the case of brain tumours, SDT can aid chemotherapy in a few ways. A major challenge associated with brain tumours is the presence of the BBB, which restricts molecules from entering the tumour site.[53] As mentioned earlier, cavitation of microbubbles can facilitate the entry of sonosensitisers into tumours.[54] By improving the selective uptake of chemotherapeutic drugs in cancer cells, SDT thereby reduces toxicity in normal cells and tissues. SDT also enhances the sensitivity of cancer cells to chemotherapeutic drugs by inducing apoptosis and inhibiting ATP-binding cassette (ABC) transporters, including ABCG2. These transporters actively efflux chemotherapeutics, which would normally lead to tumour resistance to chemotherapy. However, SDT disrupts mitochondrial function and activates the mitochondria-caspase apoptotic pathway, both of which contribute to reduced ABC transporter expression.[55] Hence, the role of US and sonosensitisers can theoretically be used alongside chemotherapy to enhance treatment efficacy. When Wang et al. investigated the potential synergistic cytotoxic effects of protoporphyrin IX (PpIX)-mediated SDT combined with chemotherapeutic drug doxorubicin (DOX), their findings demonstrated that SDT significantly enhanced the efficacy of DOX by inducing apoptosis and increasing intracellular drug uptake.[56] When SDT was coupled with DOX, the ABC transporter P-glycoprotein (P-gp) was inhibited by 21.3%, leading to an increased uptake of the drug in K562/DOX cells, a multi-drug-resistant human leukaemia cell line. While DOX alone caused apoptosis rates of 7.9%, and US and PpIX caused apoptosis rates of 13.9%, Wang et al. found that together, SDT and chemotherapy caused the highest apoptosis rate of 39.6%, suggesting the synergistic effects of SDT and chemotherapy.

SDT stems from PDT, and along with it, sonophotodynamic therapy (SPDT) emerged with the combination of SDT and PDT. PDT, however, relies on light, which, by nature, is not penetrative, while SDT relies on US waves, which can be passed through the body through water molecules. Therefore, activating sonosensitisers with a combination of US and light allows targeting tumours at different depths.[57] By activating sensitisers using both light and ultrasound simultaneously, SDPT exploits the synergistic effects of the two therapies to produce mechanical, sonochemical and photochemical activities, leading to apoptosis. Liu et al. conducted both in vitro and in vivo studies on the benefits of SPDT in human breast cancer cells and mouse mammary cancer cell lines, and found that SPDT was more effective than SDT or PDT alone.[58] In particular, while SDT alone caused a loss of cell viability of 27.36%-34.88% and PDT resulted in a loss of 36.69%-40.16% in vitro, the SPDT group exhibited a loss

of cell viability ranging from 80.49% to 85.01%. Further analysis revealed a 12.68% survival rate for SPDT-treated cancer cells, which is substantially lower than that of SDT and PDT (68.78% and 59.51%, respectively). The evident synergistic effects of SPDT can be attributed to the increased generation of ROS. Specifically, the SPDT group production of intracellular reactive oxygen species in the mice was 4-5 times more than SDT or PDT alone, suggesting that the heightened levels of ROS caused the apoptosis in SPDT. SPDT's effects on squamous cell carcinoma (SCC) tumour models were investigated.[59] While they found that PDT alone resulted in a 40% reduction in tumour volume and PDT combined with TiO_2 sensitisers achieved 55% growth inhibition, the SPDT group exhibited an 80% tumour suppression. Increased ROS generation caused this increased effectiveness; however, more electron spin resonance (ESR) measurements showed that specifically hydroxyl radicals were most prevalent in SPDT tumours, being three times higher than in SDT or PDT alone. The results of these studies indicate a synergistic effect when SDT is combined with PDT. Though PDT and SDT alone may have mild effects, when combined together, SPDT has the ability to treat deep-lying tumours that light would not reach, such as bowel and ovarian cancer or metastatic cancer that spreads to bone, lung and liver tissue.[60] SPDT is still a newly emerging combination, and its effects have still not been optimised in GBM treatment. A study found that in C6 rat models, its effects were initially promising, but its long-term impacts were less effective than PDT alone.[61] SDT surprisingly contributed to tumour growth, and a possible reason for this was that the US was incorrectly applied. Although the effects of SPDT were small, ROS production in the SPDT group was far greater than in the SDT or PDT group. Further research into sustaining SPDT's effects will be crucial for putting it into use in clinical settings.

Current Limitations and Insights of Sonodynamic Therapy

Sonosensitisers are a critical component of SDT, yet their development is still in progress. Due to a range of issues, including low stability, poor bioavailability, and selectivity, the current generation of sonosensitisers requires further research before SDT can become a more widely used treatment.[11] Some organic sonosensitisers, such as RB, for instance, have the ability to be triggered by US but are limited in their tumour accumulation ability. The lack of accumulation ability in some sensitisers has inspired research into the synthesis of their derivatives and investigations of their properties. For example, Sugita et al. investigated the development of tumour-accumulating derivatives of RB for SDT and PDT to overcome RB's poor tumour accumulation.[62] Several RB derivatives (RBD) were tested, including RBD1 (2, RB, C-2' alkyl ester, C-6 sodium salt), RBD2 (3, RB, C-2′ ω-carboxyalkyl ester, C-6 molecular form) and RBD3 (4, RB, C-2′ R-carboxyalkyl ester, C-6 molecular form). While all three derivatives behaved similarly to other sonosensitisers, RBD3 with longer alkyl chains significantly improved tumour accumulation, accumulating 1.5 times more in tumours than ATX-70 and 40 times more than RB. The researchers successfully engineered tumour-targeting RBDs by optimising amphiphilicity and showed that these modifications make RBDs promising sonosensitisers for SDT. To further combat the low selectivity of sonosensitisers, Xiong et al. isolated a novel porphyrin derivative from Photofrin known as sinoporphyrin sodium (DVDMS), which showed strong potential in SDT due to its water solubility.[63] They found that DVDMS concentrations in tumours in vivo reached approximately 98.77% and 70.37% of the maximum concentration in the tumour at 6 h and 24 h, respectively. In S180 xenografted mice, DVDMS-mediated SDT also significantly decreased tumour growth rates; It outperforms other porphyrin-based sonosensitisers due to its superior tumour selectivity, effective ROS generation and strong tumour inhibition, deeming it a promising solution, among others, to the drawbacks of current sonosensitisers.

The role of microbubbles can also be expanded in SDT. As mentioned earlier, microbubbles facilitate the activation of sonosensitisers through cavitation and pyrolysis. A promising potential of microbubbles was investigated by Nimokou et al., when they covalently attached RB to a lipid microbubble to form a conjugate, which showed heightened cytotoxicity in tumour cells and a greater reduction in tumour growth compared to RB alone.[64] First, microbubble conjugation provides site-specific, precise drug delivery, as

US selectively collapses microbubbles, activating only sonosensitisers around the tumour site. Second, the close spatial proximity of RB to the microbubble enhances the effectiveness of ROS production. In fact, when the cytotoxic potential of the MB-RB conjugate was assessed, it was found that exposure to US (1 MHz, 1.5 W/cm^2 for 30 s) decreased cell viability by 72% in RIF-1 murine fibrosarcoma cells in vitro. The results also indicated that the MB-RB conjugate had a significantly enhanced production of singlet oxygen compared to unconjugated RB, supporting the potential of MB-RB conjugates as a highly effective approach in SDT. Furthermore, microbubbles can be used to identify tumour locations more accurately: Zheng et al. developed hematoporphyrin-encapsulated poly(lactic-co-glycolic acid) microbubbles, which acted as a contrast agent for ultrasound imaging both in vitro and in vivo.[65]

Another possible function of microbubbles in SDT is to combat hypoxia levels usually associated with tumours. A compromised and anisotropic blood supply in tumours leads to inadequate oxygen and nutrient delivery to tumour cells.[66] As a result, SDT, which relies on oxygen acting as a substrate for the generation of ROS, becomes less effective and limited in its cytotoxic potential. To combat hypoxic environments, oxygen-loaded MBs (OxyMBs) conjugated with RB (OxyMB-RB) were investigated.[14] A series of in vitro and in vivo experiments was conducted. However, notable findings in the in vivo experiments revealed that OxyMB-RBs resulted in a 45% reduction in tumour volume over 5 days with US (1 MHz, 3.5 W/cm^2, 30% duty cycle, 3.5-minute exposure). In contrast, a non-oxygen-loaded MB conjugate (SF6MB-RB) resulted in a 35% increase in tumour volume. The significant difference in tumour growth between OxyMB-RB + ultrasound and SF6MB-RB + ultrasound indicates that the oxygen delivery is crucial for SDT efficacy in hypoxic tumours.

In both clinical applications and future studies, providing real-time monitoring of treatment parameters, such as ROS concentration, is crucial. Extreme concentrations of ROS generated may lead to temperatures too high, and can result in damage to surrounding healthy tissue; therefore, more research on optimising and standardising SDT parameters is beneficial.[11] A proposed insight into quantitatively measuring ROS concentration is through chemiluminescence, where probes detect the emission of light from chemical reactions occurring in SDT. Several researchers have developed chemiluminescence probes: Hu et al. developed a near-infrared area charge-coupled device which detected chemiluminescence in 2-dimensional imaging in vivo, which showed a linear relationship between singlet oxygen concentration and chemiluminescence, making quantification of ROS concentration possible in vivo.[67] Zhen et al. engineered chemiluminescent semiconducting polymer nanoparticles which were sensitive to chemiluminescent irradiation by hydrogen peroxide, and they were able to detect concentrations as low as 5 nM in vivo mouse models.[68]

Conclusion

SDT has been developed to treat various cancers, including GBM, through the generation of ROS, causing apoptosis. Numerous different sonosensitisers have been researched and tested. Currently, Photofrin II, an organic sonosensitiser, is being used in clinical applications for PDT. While it shows good antitumour effects, it is limited in its low water solubility and side effects on the patient. Inorganic sonosensitisers, however, show weaker antitumour effects but can be more easily modified to increase their efficacy. SDT has a high potential in treating GBM due to its ability to open up the BBB for a higher accumulation in tumours, especially when combined as an adjunctive treatment to other therapies, such as chemotherapy or PDT. However, more research and in vivo experimentation are needed to optimise this newly emerging therapy; it is limited by current sonosensitiser development and a lack of real-time monitoring of parameters, such as ROS concentration. Recent studies have revealed that microbubbles may have a greater role in optimising SDT since they can be conjugated to sonosensitisers to improve tumour accumulation or combat hypoxia conditions, which decrease the efficacy of SDT. Furthermore, utilising chemiluminescent probes in real time may improve the effectiveness of SDT in clinical applications, as well as enabling future studies to quantify ROS concentrations. SDT, however, will only be used in clinical applications through more experimentation and collaboration among researchers.

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Cover letter review 1:

Thank you very much for your peer review. It gave good feedback as well as actionable suggestions along with it. I have addressed your points and changed my paper accordingly.

The major changes in the paper are in the introduction. I have almost completely changed the introduction to give a better background to the audience. I have included a paragraph on GBM, its roots, and why existing therapies fail/give rise to risks. I also explain that US plays a role in enabling selective accumulation and that sonosensitisers selectively accumulate due to the enhanced permeability and retention effect. I have also added two sentences at the end of the introduction, signposting what the paper will cover.

Your second suggestion was to break up and shorten some of the longer paragraphs. I have quite significantly shortened the ‘Photofrin II’ and ‘ATX-70’ paragraphs. I have also broken up the ‘TIO2’ paragraph into three paragraphs and shortened it. I have shortened paragraphs in ‘Sonodynamic Therapy and Glioblastoma’ as well.

Your third suggestion was to discuss the risks of the treatment more. I have added a paragraph in the ‘Current limitations and Insights of SDT’, which talks about the risks of microbubble cavitation in the BBB. The risk of rupture and haemorrhage is covered, though I found that the cavitation can occur with minimal rupture if a real-time feedback-controlled system is used.

Lastly, you suggested improving the organisation of the paper. You suggested replacing the ‘Conclusion’ title with ‘Discussion’. This feels a bit strange as it would be a very short discussion, and there would be no conclusion. Instead, I named the heading ‘Sonodynamic Therapy and Glioblastoma’, which talks about SDT and its effectiveness in treating GBM, followed by a section ‘Current Limitations and Insights of SDT’.

I believe that I have covered most, if not all, of your points. Thank you very much for your invaluable feedback, and I look forward to hearing what you think of my changes. Please note that other changes were made in the paper, based on the feedback from the other reviewer.

Cover letter review 2:

Thank you very much for your review of my paper. This was extremely helpful in guiding my changes. I particularly liked the in-depth annotations you made on my paper. I have made both major and minor changes.

The introduction was the focal point of my changes: I have added a paragraph talking about glioblastoma, current treatments, and their effectiveness. I have also emphasised the need for SDT in glioblastoma, as it is shown to be more selective and less invasive. I also skim over some of its disadvantages in the introduction, with 2 sentences at the end signposting what the paper will cover later on.

At the end of 'Mechanisms in sonodynamic therapy', I have added a sentence saying: 'Sonosensitisers are another main component in SDT, and the following section presents both organic and inorganic sensitisers, as well as their advantages and drawbacks.' This makes the paper flow more nicely, as you suggested.

Finally, I have changed minor grammatical mistakes, abbreviations, and formatting from all of the annotations that you gave. I have also added the years in brackets after naming a study. All of the annotations you gave have been changed.

Again, thank you for this excellent review, which led to the change of certain parts of the paper. Please do note that other changes were made (both major and minor) from the other peer review. This included shortening/breaking up paragraphs and discussing the risks of SDT more. I look forward to your thoughts on my changes.

REVIEW ARTICLE

Sonodynamic Therapy in the Treatment of Glioblastoma: Mechanisms, Challenges, and Insights.

[Name redacted by Managing Editor]

[School redacted by Managing Editor]

[Email redacted by Managing Editor]

Abstract

Sonodynamic therapy is a rapidly evolving approach in cancer treatment, which uses ultrasound waves and sonosensitisers, drugs which are sensitive to ultrasound. Stemming from photodynamic therapy, it works by exposing sonosensitisers in tumour cells to high-intensity ultrasound waves, generating reactive oxygen species that cause damage to tumour cells. Among existing therapies, it is minimally invasive with fewer side effects and has the potential to enhance treatment efficacy, especially when combined with other strategies as a secondary treatment. Glioblastoma multiforme has proven to be a hard-to-treat cancer, with many existing treatments having limited efficacy against it due to the presence of the blood-brain barrier. This novel adjunctive treatment is promising in the context of glioblastoma multiforme because it is less invasive than other treatments and potentially more effective in overcoming blood-brain barrier limitations. Nevertheless, sonodynamic therapy relies on sonosensitisers, which are currently limited in their efficacy, and the lack of real-time monitoring of parameters in this treatment can lead to uncontrolled cytotoxic effects. This paper addresses the mechanisms of sonodynamic therapy, its application in combination with other therapies, its disadvantages and, more importantly, suggests solutions to its drawbacks.

Keywords

Translational Medical Sciences; Disease Treatment and Therapies; Sonodynamic Therapy; Ultrasound; Glioblastoma Multiforme

Introduction

Cancer is characterised by the uncontrolled growth of cells, which may be able to invade nearby tissues. Over the past decades, cancer has become the second leading cause of global mortality.[1] Although current cancer treatment methods, such as chemotherapy, radiotherapy, immunotherapy and targeted therapies, have proven to be reliable, they are not always curative.

GBM is the most aggressive and common form of primary brain cancer in adults, classified as a Grade IV tumour by the WHO.[2] It can either arise de novo (primary GBM) or from lower-grade gliomas (secondary GBM). The main treatments for GBM currently are chemotherapy, surgery and radiotherapy. While Temozolomide chemotherapy shows signs of success, its median survival time is only 16 months.[3] Major drawbacks of chemotherapy include its toxicity and side effects, such as myelosuppression and fatigue, as well as chemoresistance. As a result of chemotherapy's ineffectiveness, most patients relapse. Radiotherapy also shows limited efficacy in GBM treatment. Approximately 90% of GBM recurrences occur within 2 cm of the original tumour site post-radiotherapy, suggesting that it does not prevent the spread of the tumour.[4] Standard radiotherapy showed a median survival of 8-10 months, showing signs of low effectiveness. Chemotherapy and radiation therapy have poor tumour selectivity and can lead to increased therapy resistance through enhanced DNA repair mechanisms and hypoxia-inducible factor 1.[5] This occurs when they cause hypoxic conditions in tumour cells, creating a pro-angiogenic and pro-stemness environment, which leads to therapeutic resistance. Immunotherapy has also proven to be ineffective against GBM due to the strong immunosuppressive tumour microenvironment of GBM tumours

as well as the blood-brain barrier (BBB), which is impermeable to large molecules and immune cells.[6] Its heterogeneous nature and the protection from the BBB make it especially resistant to targeted therapies.[7,8] The standard treatment for glioblastoma consists of surgery followed by radiotherapy and/or chemotherapy.[9] However, glioblastomas grow rapidly and are fatal. In treating GBM, existing anticancer strategies have relatively low effectiveness, with the five-year rate of survival for patients being only 6.9%.[10–12]

Sonodynamic therapy (SDT) is a novel cancer treatment which is minimally invasive.[13] It works by activating pre-administered sonosensitive drugs with ultrasound (US) and generating reactive oxygen species (ROS), which are responsible for cell damage. The US plays a role in temporarily opening up the BBB, enabling larger molecules to enter the tumour more easily. The enhanced permeability and retention effect (EPR) enables sonosensitisers to selectively accumulate, making it less invasive than other therapies.[14] SDT was inspired by photodynamic therapy (PDT), a method of treatment using visible light to induce tumour necrosis. In 1989, several photosensitisers, which were hematoporphyrin derivatives (HPDs), were found to be sensitive to US and had the ability to induce cell damage when activated.[15] Compared to using visible light to induce tumour necrosis, US was shown to be more effective, as a study found that sonodynamic therapy inhibited tumour growth in murine squamous cell carcinoma in mice by 77%.[16] In contrast, photodynamic therapy caused tumour growth inhibition of only 27%. Light waves cannot penetrate beyond a few millimetres of soft tissue, while high-intensity focused ultrasound (HIFU) has a penetration depth of up to 12 cm in soft tissue.[17,18]

Despite its potential advantages, SDT faces obstacles that must be overcome before it is fully recognised as a secondary treatment. Current sonosensitisers all have drawbacks, including poor bioavailability and selectivity, low reactive oxygen species (ROS) yield, and post-treatment side effects.[19] Another challenge associated with SDT is the lack of real-time monitoring of ROS and temperature. Too high temperatures may cause excessive production of ROS, leading to unwanted cell damage, whereas temperatures that are too low may not produce sufficient ROS needed to cause apoptosis. Therefore, it is crucial to monitor ROS concentration and temperature during treatment.[20]

This paper will cover the primary mechanisms which enable SDT to function, as well as evaluate the important types of sonosensitisers available. The challenges associated with SDT will be discussed along with its potential solutions, through reviewing their past and current approaches.

Mechanisms in Sonodynamic Therapy

The primary way that SDT leads to cell death is the production of ROS when a sonosensitiser is ‘activated’ as a result of microbubble cavitation, which occurs when sound waves at a certain frequency range permeate through aqueous environments.[21] The first step in the mechanism involves the cavitation of microbubbles. Microbubbles are microscopic air/oxygen bubbles ranging from 1-100 μm, which have great potential in site-specific drug delivery.[22] These microbubbles already exist in aqueous environments, but can be deliberately administered intravenously to patients to increase the efficacy of SDT.[23] Under US exposure, they undergo cyclic expansion and contraction in a process known as cavitation, which induces rapid temperature and pressure changes. Cavitation can either be ‘stable’ or ‘inertial’, where microbubbles experience violent, uncontrolled oscillations at higher acoustic pressures.[24]

Inertial cavitation can subsequently cause sonosensitisers to rise to a higher energy state, leading to cytotoxicity through two possible mechanisms: sonoluminescence and pyrolysis. Sonoluminescence occurs when collapsing microbubbles release energy in the form of visible light.[25] Since most sonosensitisers are derived from photosensitisers, they are sensitive to light and will shift from their ground state to an excited state with higher energy. As they return to the ground state, they release energy to surrounding oxygen molecules to produce ROS, such as singlet oxygen, superoxide and hydrogen peroxide, which can damage intracellular DNA, promote lipid peroxidation, and result in apoptosis to targeted tumour cells, as shown in **Figure 1**.[26] Pyrolysis is similar in many ways; the only difference is that microbubbles shift sonosensitisers to an excited state by generating extreme amounts of

thermal energy, rather than light. It is believed that inertial cavitation produces shockwave effects which thermally dissociate water vapour into hydroxyl radicals and hydrogen radicals even without the presence of a sonosensitiser, which leads to cell death.[27]

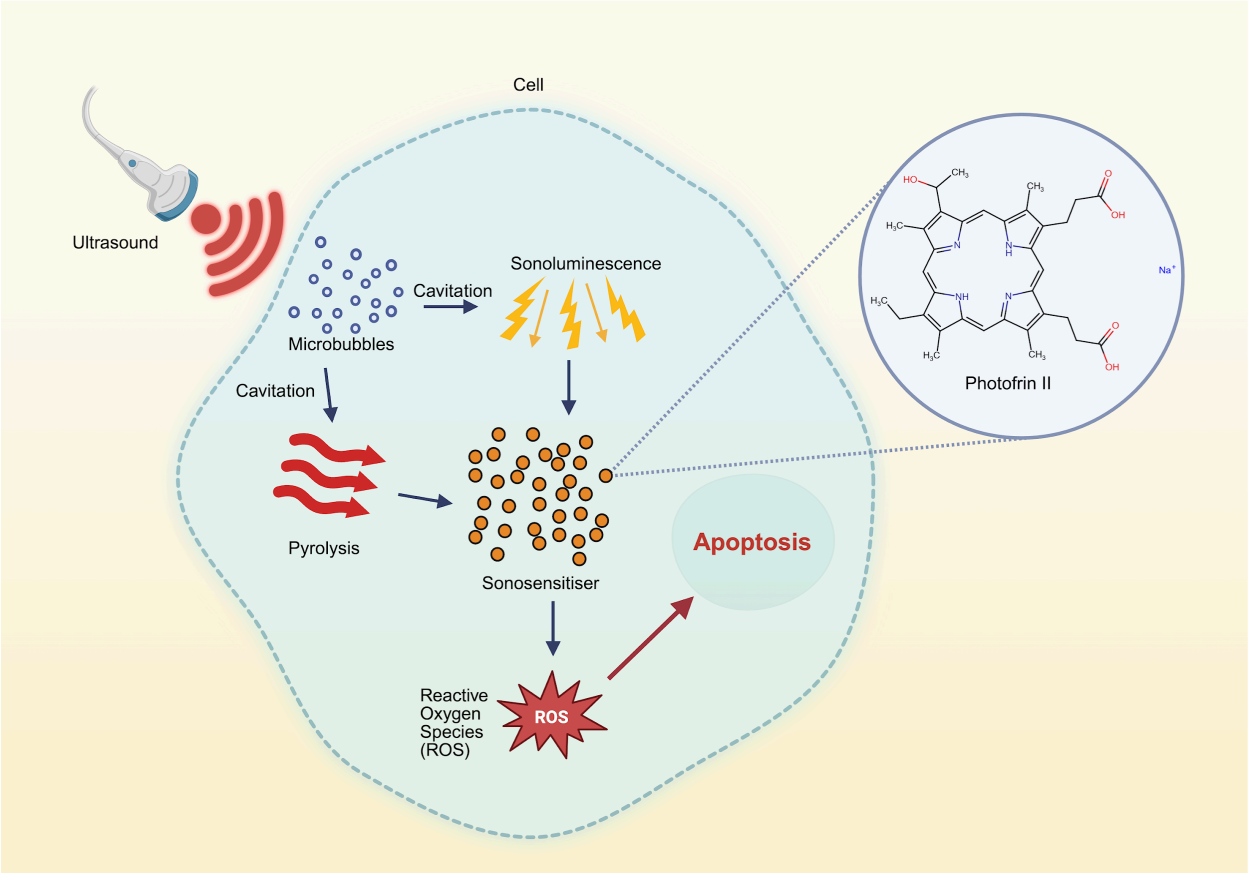

Figure 1: Schematic diagram to show microbubble cavitation leading to the generation of reactive oxygen species through sonoluminescence and pyrolysis, with Photofrin II as an example of a sonosensitiser. Figure created using https://BioRender.com and https://chemaxon.com/.

Microbubbles have a greater role than cavitation alone; they can enhance BBB permeability to aid drug entry into tumours and increase tumour specificity. This is especially useful in GBM, where the presence of the BBB would otherwise prevent certain drugs from entering. The BBB is a highly selective barrier formed by endothelial cells surrounding the brain's capillaries. It controls which substances can pass from the blood into the brain, shielding the central nervous system from toxins, pathogens, and chemicals.[28] In fact, the BBB blocks all large molecules and 98% of micro-molecule drugs from entering the tumour.[29] However, microbubble cavitation can cause significant shear stress on tumour cells and create ruptures in cell membranes in a process called cell lysis, thus enhancing the permeability of the tumour for sonosensitisers.[30,31] Another possible way microbubbles enhance permeability in tumour cells is through the generation of ROS, as mentioned earlier. Even without a sonosensitiser, microbubbles can generate ROS when in contact with US through pyrolysis, which is another possible explanation for increased tumour permeability.[32]

Furthermore, microbubbles can improve targeted drug release. Glioma tumours are rich in vascular endothelial growth factor (VEGF). Fan et al. (2013) designed VEGF-conjugated BCNU-loaded microbubbles, which specifically bind to VEGF receptor two overexpressed in GBM tumours, thus allowing localised drug release at the tumour site and reducing damage to healthy tissue.[33] This indicates

that microbubbles further increase the efficacy of SDT by targeted treatment and increased permeability in the tumour. Sonosensitisers are another main component in SDT, and the following section presents both organic and inorganic sensitisers, as well as their advantages and drawbacks.

Organic Sonosensitisers

Sonosensitisers have a greater ability to selectively accumulate in tumour sites due to the enhanced permeability and retention (EPR) effect in tumours.[14] When Yumita et al. (1989) found that several HPDs used in PDT were sensitive to US, they became the main focal point in sonosensitiser development, with Photofrin II and ATX-70 inducing severe cell damage when activated by US.[34,35] Organic sonosensitisers are mainly composed of carbon, particularly with carbon-hydrogen and carbon-carbon bonds. Despite having relatively good tumour specificity and ROS generation ability, even these commonly researched organic sonosensitisers have their downsides.

Photofrin II

Photofrin II is a family of oligomers where multiple hematoporphyrin molecules are bonded together, with the ability to retain in tumours longer than in healthy tissue selectively and has been FDA-approved as a photosensitiser in cancer PDT.[36] A study on the effects of Photofrin II compared the survival rates of leukemic (MT-2) and normal peripheral mononuclear cells (PMNC). Using ultrasound (450 kHz, 0.3-0.5 W/cm^2), MT-2 cell survival decreased with higher sensitiser concentrations, while healthy cells showed no difference in survival rate between groups with and without Photofrin II administration. In patients with acute-type adult T cell leukaemia, PMNC survival decreased from 69.4% ± 22.5% with ultrasound alone to 30.0% ± 23.0% with Photofrin.[37] Although survival rates of leukemic cells are still relatively high, Photofrin II dropped leukemic cell survival rates by half. At the same time, healthy tissue showed no difference in survival rates, suggesting that Photofrin II has minimal toxicity to healthy cells. However, since porphyrins are photosensitive, they may cause post-administration side effects, including skin sensitivity to light and external burns, as shown in **Table 1.** Furthermore, their low water solubilities call for the use of nanocarriers to enhance accumulation in tumour tissues.[38]

ATX-70

ATX-70 is a gallium porphyrin complex which showed the highest accumulation concentration in tumours out of the HPDs tested in this study by Nakajima et al. (1990). It also exhibits the longest phosphorescence lifetime among HPDs.[39] In other words, ATX-70 absorbs and reemits sonoluminescent light the longest while also selectively accumulating in tumours. The efficacy of ATX-70 was studied using isolated sarcoma 180 cells, and findings demonstrated that an 80 μM concentration of ATX-70 increased the rate of damage to the mice sarcoma by four times under 2 MHz US, compared to the two times increased rate with hematoporphyrin.[40] This suggests that ATX-70 significantly amplifies US-induced cytotoxic effects, primarily through the generation of singlet oxygen radicals, and potentially has a greater effect than Photofrin II and other HPDs. The in vivo effects of ATX-70 in Sprague-Dawley rats with mammary tumours were examined and showed that peak accumulation of ATX-70 in tumours was 24 hours post-administration, similar to Photofrin II.[41] 2 weeks after administration of ATX-70 and US radiation (3 W/cm^2), there was a mean reduction of tumour volume of 50%. With radiation of 5 W/cm^2, a further 18% reduction in tumour volume was observed compared to the control group (no treatment). ATX–70–mediated sonodynamic therapy significantly enhanced tumour regression and confirmed its efficacy as a sonosensitiser. Nevertheless, ATX-70 is a porphyrin derivative and shares the same downsides as Photofrin II, such as photosensitivity and low water solubility. Yumita et al. (2000) tested another HPD, known as ATX-S10, and found similar results to ATX-70 and Photofrin II, suggesting that HPDs share common characteristics and effects as a sonosensitiser.[42]

Rose Bengal

Another organic compound, Rose Bengal (RB), a xanthene dye, has also been tested for its potential cytotoxic effects when exposed to US. Umemura et al. (1999) have shown that RB increased ultrasound-induced cell damage to isolated sarcoma 180 cells by a factor of 2-3 at a concentration of 160 μM.[43] At a US exposure of 5.9 W/cm², cell viability dropped to as low as 4% in the presence of 160 μM RB, but only to 38% with US alone after 60 seconds. However, after comparing the results of this study to results of previous studies, it was observed that to achieve similar cytotoxic effects, RB concentration needed to be at least double the concentration of porphyrins, suggesting that porphyrins have a slight advantage in efficacy in terms of cell damage.[21,34,40] Another drawback of xanthene dyes is that the liver can easily capture and break them down, making them unable to accumulate in tumours.[44]

Inorganic Sonosensitisers

Titanium dioxide (TiO_2) nanoparticles

Inorganic sonosensitisers are substances that are not primarily composed of carbon and hydrogen in a chain. They fall into two categories: metal and non-metal. Titanium dioxide (TiO_2) nanoparticles (NPs) are a class of metal-based sonosensitisers that also produce ROS when activated by ultrasound.[45] As a superconducting material, it can produce electron-hole pairs when excited by ultrasound.[46] These pairs interact with nearby oxygen and water molecules to produce hydroxyl radicals and superoxide anions. An in vitro study suggested that TiO_2 NPs or US alone had little effect on mouse melanoma C32 cells, with cell viability remaining above 92% after US exposure at intensities up to 1.0 W/cm^2 for 10 seconds. However, combining a TiO_2 solution (0.500% w/w) with US at 1.0 W/cm² for 10 seconds resulted in a decrease in cell viability to 53.6 ± 1.8%, and apoptotic cell levels were 2.73 times higher than those in the control group (no treatment). Additionally, microscopic analysis confirmed that the TiO_2 particles were responsible for cell membrane damage, indicating the production of ROS.[47]

Despite its potential apoptosis-inducing ability, TiO_2 NPs tend to aggregate in physiological environments, which enables them to be easily captured by the reticuloendothelial system.[45] Therefore, research on carrier systems that can improve the dispersion stability of TiO_2 NPs under physiological pH conditions will significantly enhance the efficacy of TiO_2 NPs as sonosensitisers in SDT. You et al. (2016) encapsulated TiO_2 NPs in carboxymethyl dextran (CMD). This long-circulating hydrophilic TiO_2 NP still retained the ability to produce ROS when activated by US, but improved systemic circulation and uptake into cells.[48] Another study used an encapsulation of TiO_2 NPs in polyion complex micelles to improve their dispersion stability while producing singlet oxygen.[49]

TiO_2 NPs possess another drawback, namely, electron-hole recombination: As mentioned earlier, TiO_2 produces electrons and holes upon exposure to ultrasound. However, electrons and holes can recombine quite easily, resulting in reduced ROS production.[46] Other metal-based sonosensitisers that include Manganese-based composites and Fe_3O_4-loaded sensitisers also show antitumour effects, but may be limited in the complexity of their design and their reduced ROS production rate under non-acidic and hypoxic conditions. Therefore, more research should be done on overcoming these obstacles.[45]

Silicon nanostructures

Non-metal sonosensitisers have also been widely used in SDT research. Silicon nanostructures, for instance, can cause amplified sonodynamic effect, making them a potential sonosensitiser. A study found that silicon nanoplatforms (SiNPs) had a significant cytotoxic effect when paired with US: While US (0.88 MHz, 0.05 W/cm^2) alone only led to 50% Hep-2 cancer cell death in vitro, when combined with SiNPs (0.2 mg/ml), 90% of the cancer cells were destroyed.[50] The researchers also conducted in vivo experiments on mice with Lewis lung carcinoma (LLC). The mice received injections of a SiNP suspension (1 mg/ml) into the tumour. When exposed to US, tumour growth was inhibited by 30 ± 5% over the course of 13 days, further suggesting that SiNP may be a possible sonosensitiser in SDT. Furthermore, Mesoporous silica nanoparticles (MSNs) have been modified in the past to alter their hydrophilicity, enabling microbubbles to

be trapped in a hydrophobic MSN and increasing ROS production.[45] However, despite showing some promise in vitro and in vivo, the efficacy and safety of SiNP in human clinical trials remain unclear.[51]

Type	Sonosensitiser	Advantages	Disadvantages
Organic	Photofrin II	• FDA-approved for PDT.[36] • Significant tumour growth inhibition in vivo.[35,37] • Good accumulation in tumours.[36]	• High phototoxicity.[38] • Low water solubility.[38]
Organic	ATX-70	• Long phosphorescence lifetime.[39] • Significant tumour growth inhibition in vivo.[39,41,52,53] • Good accumulation in tumours.[39]	• High phototoxicity.[38] • Low water solubility.[38]
Organic	Rose Bengal (RB)	• Good tumour growth inhibition.[43]	• Requires higher concentration than porphyrins.[20,34,40] • Easily captured by the liver.[44] • Poor accumulation in tumours.[44]
Inorganic	Titanium dioxide nanoparticles (TiO_2 NPs)	• Good tumour growth inhibition.[45–47] • Can be modified to improve circulation and ROS production.[48,49]	• Aggregates and can be captures by the reticuloendothelial system.[45,48,49] • Electron-hole recombination reduces ROS production.[46]
Inorganic	Silicon nanoparticles (SiNPs)	• Medium tumour growth inhibition.[50] • Hydrophobic SiNPs can carry microbubbles to increase ROS production.[45]	• Antitumour effects are not as strong as those of porphyrins.[50]

Table 1: Advantages and disadvantages of inorganic and organic sonosensitisers.

Sonodynamic Therapy and Glioblastoma

In vitro experiments have demonstrated the potential of SDT in combating GBM. 5-Aminolevulinic acid (5-ALA), a precursor of porphyrins, has been extensively researched for its application in treating GBM. 5-ALA, at concentrations of 10 μm/mL, coupled with US at a power of 6 W, significantly reduced cell viability in rat RG2 glioma cells in vitro.[54] Similar observations were found in mouse cells in vitro as well as human U87 GBM cells.[55,56] Other sonosensitisers, including RB, sinoporphyrin sodium and PpIX have

also shown apoptotic effects in glioma cells.[57–59] Though SDT may not be a likely primary treatment in the future, it has high potential to become a supporting treatment. To date, research has shown that SDT can be combined with other therapies such as chemotherapy and PDT to yield synergistic effects.

Chemotherapy, as mentioned earlier, is a mainstream therapy used in GBM treatment. In the case of brain tumours, SDT can aid chemotherapy in a few ways. A major challenge associated with brain tumours is the presence of the BBB, which restricts molecules from entering the tumour site.[60] As mentioned earlier, cavitation of microbubbles can facilitate the entry of sonosensitisers into tumours.[61] By improving the selective uptake of chemotherapeutic drugs in cancer cells, SDT thereby reduces toxicity in normal cells and tissues.[62] SDT also enhances the sensitivity of cancer cells to chemotherapeutic drugs by inducing apoptosis and inhibiting ATP-binding cassette (ABC) transporters, including ABCG2. These transporters actively efflux chemotherapeutics, which would normally lead to tumour resistance to chemotherapy. However, SDT disrupts mitochondrial function and activates the mitochondria-caspase apoptotic pathway, both of which contribute to reduced ABC transporter expression.[63] Hence, the role of US and sonosensitisers can theoretically be used alongside chemotherapy to enhance treatment efficacy. When Wang et al. (2015) investigated the potential synergistic cytotoxic effects of PpIX-mediated SDT combined with the chemotherapeutic drug doxorubicin (DOX), their findings demonstrated that SDT significantly enhanced the efficacy of DOX by inducing apoptosis and increasing intracellular drug uptake.[62] When SDT was coupled with DOX, the ABC transporter P-glycoprotein (P-gp) was inhibited by 21.3%, leading to an increased uptake of the drug in K562/DOX cells, a multi-drug-resistant human leukaemia cell line. While DOX alone caused apoptosis rates of 7.9%, and US and PpIX caused apoptosis rates of 13.9%, Wang et al. (2015) found that together, SDT and chemotherapy caused the highest apoptosis rate of 39.6%, suggesting the synergistic effects of SDT and chemotherapy.

SDT stems from PDT, and along with it, sonophotodynamic therapy (SPDT) emerged with the combination of SDT and PDT. PDT, however, relies on light, which, by nature, is not penetrative, while SDT relies on US waves, which can be passed through the body through water molecules. US waves propagate through water molecules through a series of compressions and rarefactions. Therefore, activating sonosensitisers with a combination of US and light allows targeting tumours at different depths.[65] photosensitisers can be excited by using light with a certain wavelength, much like sonosensitisers with sound.[66] By activating sensitisers using both light and ultrasound simultaneously, SDPT exploits the synergistic effects of the two therapies to produce mechanical, sonochemical and photochemical activities, leading to apoptosis. Liu et al. (2016) conducted both in vitro and in vivo studies on the benefits of SPDT in human breast cancer cells and mouse mammary cancer cell lines, and found that SPDT was more effective than SDT or PDT alone.[67] In particular, while SDT alone caused a loss of cell viability of 27.36%-34.88% and PDT resulted in a loss of 36.69%-40.16% in vitro, the SPDT group exhibited a loss of cell viability ranging from 80.49% to 85.01%. Further analysis revealed a 12.68% survival rate for SPDT-treated cancer cells, which is substantially lower than that of SDT and PDT (68.78% and 59.51%, respectively). The evident synergistic effects of SPDT can be attributed to the increased generation of ROS. Specifically, the SPDT group produced intracellular ROS in mice 4-5 times more than SDT or PDT alone, suggesting that the heightened levels of ROS caused apoptosis in SPDT.

SPDT's effects on squamous cell carcinoma (SCC) tumour models were investigated.[68] While it was found that PDT alone resulted in a 40% reduction in tumour volume and PDT combined with TiO_2 sensitisers achieved 55% growth inhibition, the SPDT group exhibited an 80% tumour suppression. Increased ROS generation caused this increased effectiveness; however, more electron spin resonance (ESR) measurements showed that specifically hydroxyl radicals were most prevalent in SPDT tumours, being three times higher than in SDT or PDT alone. The results of these studies indicate a synergistic effect when SDT is combined with PDT. Though PDT and SDT alone may have mild effects, when combined together, SPDT has the ability to treat deep-lying tumours that light would not reach, such as bowel and ovarian cancer, or metastatic cancer that spreads to bone, lung and liver tissue.[69] SPDT is still a newly emerging combination, and its effects have still not been optimised in GBM treatment. A study found that in C6 rat models, its effects were initially promising, but its long-term impacts were less

effective than PDT alone.[70] SDT surprisingly contributed to tumour growth, and a possible reason for this was that the US was incorrectly applied. Although the effects of SPDT were small, ROS production in the SPDT group was far greater than in the SDT or PDT group.[70] Further research into sustaining SPDT's effects will be crucial for putting it into use in clinical settings.

Current Limitations and Insights of Sonodynamic Therapy

Sonosensitisers are a critical component of SDT, yet their development is still in progress. Due to a range of issues, including low stability, poor bioavailability, and selectivity, the current generation of sonosensitisers requires further research before SDT can become a more widely used treatment.[20] Some organic sonosensitisers, such as RB, for instance, have the ability to be triggered by US but are limited in their tumour accumulation ability. The lack of accumulation ability in some sensitisers has inspired research into the synthesis of their derivatives and investigations of their properties. For example, Sugita et al. (2007) investigated the development of tumour-accumulating derivatives of RB for SDT and PDT to overcome RB's poor tumour accumulation.[71] Several RB derivatives (RBD) were tested, including RBD1 (2, RB, C-2' alkyl ester, C-6 sodium salt), RBD2 (3, RB, C-2′ ω-carboxyalkyl ester, C-6 molecular form) and RBD3 (4, RB, C-2′ R-carboxyalkyl ester, C-6 molecular form). While all three derivatives behaved similarly to other sonosensitisers, RBD3 with longer alkyl chains significantly improved tumour accumulation, accumulating 1.5 times more in tumours than ATX-70 and 40 times more than RB. The researchers successfully engineered tumour-targeting RBDs by optimising amphiphilicity and showed that these modifications make RBDs promising sonosensitisers for SDT.[71] To further combat the low selectivity of sonosensitisers, Xiong et al. (2015) isolated a novel porphyrin derivative from Photofrin known as sinoporphyrin sodium (DVDMS), which showed strong potential in SDT due to its water solubility.[72] The results of this study demonstrated that DVDMS concentrations in tumours in vivo reached approximately 98.77% and 70.37% of the maximum concentration in the tumour at 6 h and 24 h, respectively. In S180 xenografted mice, DVDMS-mediated SDT also significantly decreased tumour growth rates;[72] It outperforms other porphyrin-based sonosensitisers due to its superior tumour selectivity, effective ROS generation and strong tumour inhibition, deeming it a promising solution, particularly when compared to the current sonosensitisers and their individual disadvantages.

The role of microbubbles can also be expanded in SDT. As mentioned earlier, microbubbles facilitate the activation of sonosensitisers through cavitation and pyrolysis. A promising potential of microbubbles was investigated by Nimokou et al. (2012), when they covalently attached RB to a lipid microbubble to form a conjugate, which showed heightened cytotoxicity in tumour cells and a greater reduction in tumour growth compared to the use of RB alone.[73] First, microbubble conjugation provides site-specific, precise drug delivery, as US selectively collapses microbubbles, activating only sonosensitisers around the tumour site. Second, the close spatial proximity of RB to the microbubble enhances the effectiveness of ROS production. In fact, when the cytotoxic potential of the MB-RB conjugate was assessed, it was found that exposure to US (1 MHz, 1.5 W/cm^2 for 30 s) decreased cell viability by 72% in RIF-1 murine fibrosarcoma cells in vitro.[73] The results also indicated that the MB-RB conjugate had a significantly enhanced production of singlet oxygen compared to unconjugated RB, supporting the potential of MB-RB conjugates as a highly effective approach in SDT. Furthermore, microbubbles can be used to identify tumour locations more accurately: Zheng et al. (2012) developed hematoporphyrin-encapsulated poly(lactic-co-glycolic acid) microbubbles, which acted as a contrast agent for ultrasound imaging both in vitro and in vivo.[74] This suggests that microbubbles may have a greater use, not only in SDT delivery, but also in cancer diagnosis.

Another possible function of microbubbles in SDT is to combat hypoxia levels usually associated with tumours. A compromised and anisotropic blood supply in tumours leads to inadequate oxygen and nutrient delivery to tumour cells.[75] As a result, SDT, which relies on oxygen acting as a substrate for the

generation of ROS, becomes less effective and limited in its cytotoxic potential. To combat hypoxic environments, oxygen-loaded MBs (OxyMBs) conjugated with RB (OxyMB-RB) were investigated.[23] A series of in vitro and in vivo experiments was conducted. However, notable findings in the in vivo experiments revealed that OxyMB-RBs resulted in a 45% reduction in tumour volume over 5 days with US (1 MHz, 3.5 W/cm^2, 30% duty cycle, 3.5-minute exposure). In contrast, a non-oxygen-loaded MB conjugate (SF6MB-RB) resulted in a 35% increase in tumour volume. The significant difference in tumour growth between OxyMB-RB + ultrasound and SF6MB-RB + ultrasound indicates that the oxygen delivery is crucial for SDT efficacy in hypoxic tumours.[23]

As stated earlier, the cavitation effect of microbubbles can temporarily open up the BBB and improve drug uptake by tumours in the brain. However, the ability of US to open the BBB also poses risks. Excessive exposure of microbubbles to focused US can significantly reduce the energy threshold for brain lesion creation, causing vascular injury and haemorrhage. [76] Compromising the BBB could allow cells to escape the tumour and may lead to an increased risk of metastasis. However, under a certain threshold, there is the possibility of a 100% BBB opening rate without any haemorrhage or vascular damage.[77] More research and in vivo studies are needed to verify the effects of microbubble cavitation on the BBB. To mitigate the possibility of rupture and haemorrhage, O'Reilly and Hynynen (2012) tested a real-time feedback-controlled focused US system which monitors inertial cavitation in the body and adjusts the ultrasound pressure according to the feedback data. They found that this system could be a reliable way to prevent excessive exposure to US, ensuring the BBB remains open temporarily with minimal rupture and vascular damage.[78]

In both clinical applications and future studies, providing real-time monitoring of treatment parameters, such as ROS concentration, is crucial. Extreme concentrations of ROS generated may lead to temperatures too high, and can result in damage to surrounding healthy tissue; therefore, more research on optimising and standardising SDT parameters is beneficial.[20] A proposed insight into quantitatively measuring ROS concentration is through chemiluminescence, where probes detect the emission of light from chemical reactions occurring in SDT. Several researchers have developed chemiluminescence probes: Hu et al. (2011) developed a near-infrared area charge-coupled device, which detected chemiluminescence in 2-dimensional imaging in vivo, showing a linear relationship between singlet oxygen concentration and chemiluminescence, making quantification of ROS concentration possible in vivo.[79] Zhen et al. (2016) engineered chemiluminescent semiconducting polymer nanoparticles which were sensitive to chemiluminescent irradiation by hydrogen peroxide, and they were able to detect concentrations as low as 5 nM in vivo mouse models.[80]

Conclusion

SDT has been developed to treat various cancers, including GBM. SDT starts by inducing microbubble cavitation through US, leading to ROS generation from sonosensitisers. ROS is responsible for cell damage and apoptosis of tumours. Numerous sonosensitisers have been researched and tested. Currently, Photofrin II, an organic sonosensitiser, is being used in clinical applications for PDT. While it shows good antitumour effects, it is limited in its low water solubility and side effects on the patient. Inorganic sonosensitisers, however, show weaker antitumour effects but can be more easily modified to increase their efficacy. SDT has a high potential in treating GBM due to its ability to open up the BBB for a higher accumulation in tumours, especially when combined as an adjunctive treatment to other therapies, such as chemotherapy or PDT. However, more research and in vivo experimentation are needed to optimise this newly emerging therapy; it is limited by current sonosensitiser development and a lack of real-time monitoring of parameters, such as ROS concentration. Recent studies have revealed that microbubbles may have a greater role in optimising SDT since they can be conjugated to sonosensitisers to improve tumour accumulation or combat hypoxia conditions, which decrease the efficacy of SDT. Furthermore, utilising chemiluminescent probes in real time may improve the effectiveness of SDT in clinical applications, as well as enabling future studies to quantify ROS concentrations. SDT, however, will only be used in clinical applications through more experimentation and collaboration among researchers.

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REVIEW ARTICLE

Sonodynamic Therapy in the Treatment of Glioblastoma: Mechanisms, Challenges, and Insights.

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Abstract

Sonodynamic therapy is a rapidly evolving approach in cancer treatment, which uses ultrasound waves and sonosensitisers, drugs which are sensitive to ultrasound. Stemming from photodynamic therapy, it works by exposing sonosensitisers in tumour cells to high-intensity ultrasound waves, generating reactive oxygen species that cause damage to tumour cells. Among existing therapies, it is minimally invasive with fewer side effects and has the potential to enhance treatment efficacy, especially when combined with other strategies as a secondary treatment. Glioblastoma multiforme has proven to be a hard-to-treat cancer, with many existing treatments having limited efficacy against it due to the presence of the blood-brain barrier. This novel adjunctive treatment is promising in the context of glioblastoma multiforme because it is less invasive than other treatments and potentially more effective in overcoming blood-brain barrier limitations. Nevertheless, sonodynamic therapy relies on sonosensitisers, which are currently limited in their efficacy, and the lack of real-time monitoring of parameters in this treatment can lead to uncontrolled cytotoxic effects. This paper addresses the mechanisms of sonodynamic therapy, its application in combination with other therapies, its disadvantages and, more importantly, suggests solutions to its drawbacks.

Keywords

Translational Medical Sciences; Disease Treatment and Therapies; Sonodynamic Therapy; Ultrasound; Glioblastoma Multiforme

Introduction

Cancer is characterised by the uncontrolled growth of cells, which may be able to invade nearby tissues. Over the past decades, cancer has become the second leading cause of global mortality.[1] Although current cancer treatment methods, such as chemotherapy, radiotherapy, immunotherapy and targeted therapies, have proven to be reliable, they are not always curative.

GBM is the most aggressive and common form of primary brain cancer in adults, classified as a Grade IV tumour by the WHO.[2] It can either arise de novo (primary GBM) or from lower-grade gliomas (secondary GBM). The main treatments for GBM currently are chemotherapy, surgery and radiotherapy. While Temozolomide chemotherapy shows signs of success, its median survival time is only 16 months.[3] Major drawbacks of chemotherapy include its toxicity and side effects, such as myelosuppression and fatigue, as well as chemoresistance. As a result of chemotherapy's ineffectiveness, most patients relapse. Radiotherapy also shows limited efficacy in GBM treatment. Approximately 90% of GBM recurrences occur within 2 cm of the original tumour site post-radiotherapy, suggesting that it does not prevent the spread of the tumour.[4] Standard radiotherapy showed a median survival of 8-10 months, showing signs of low effectiveness. Chemotherapy and radiation therapy have poor tumour selectivity and can lead to increased therapy resistance through enhanced DNA repair mechanisms and hypoxia-inducible factor 1.[5] This occurs when they cause hypoxic conditions in tumour cells, creating a pro-angiogenic and pro-stemness environment, which leads to therapeutic resistance. Immunotherapy has also proven to be ineffective against GBM due to the strong immunosuppressive tumour microenvironment of GBM tumours

as well as the blood-brain barrier (BBB), which is impermeable to large molecules and immune cells.[6] Its heterogeneous nature and the protection from the BBB make it especially resistant to targeted therapies.[7,8] The standard treatment for glioblastoma consists of surgery followed by radiotherapy and/or chemotherapy.[9] However, glioblastomas grow rapidly and are fatal. In treating GBM, existing anticancer strategies have relatively low effectiveness, with the five-year rate of survival for patients being only 6.9%.[10–12]

Sonodynamic therapy (SDT) is a novel cancer treatment which is minimally invasive.[13] It works by activating pre-administered sonosensitive drugs with ultrasound (US) and generating reactive oxygen species (ROS), which are responsible for cell damage. The US plays a role in temporarily opening up the BBB, enabling larger molecules to enter the tumour more easily. The enhanced permeability and retention effect (EPR) enables sonosensitisers to selectively accumulate, making it less invasive than other therapies.[14] SDT was inspired by photodynamic therapy (PDT), a method of treatment using visible light to induce tumour necrosis. In 1989, several photosensitisers, which were hematoporphyrin derivatives (HPDs), were found to be sensitive to US and had the ability to induce cell damage when activated.[15] Compared to using visible light to induce tumour necrosis, US was shown to be more effective, as a study found that sonodynamic therapy inhibited tumour growth in murine squamous cell carcinoma in mice by 77%.[16] In contrast, photodynamic therapy caused tumour growth inhibition of only 27%. Light waves cannot penetrate beyond a few millimetres of soft tissue, while high-intensity focused ultrasound (HIFU) has a penetration depth of up to 12 cm in soft tissue.[17,18]

Despite its potential advantages, SDT faces obstacles that must be overcome before it is fully recognised as a secondary treatment. Current sonosensitisers all have drawbacks, including poor bioavailability and selectivity, low reactive oxygen species (ROS) yield, and post-treatment side effects.[19] Another challenge associated with SDT is the lack of real-time monitoring of ROS and temperature. Too high temperatures may cause excessive production of ROS, leading to unwanted cell damage, whereas temperatures that are too low may not produce sufficient ROS needed to cause apoptosis. Therefore, it is crucial to monitor ROS concentration and temperature during treatment.[20]

This paper will cover the primary mechanisms which enable SDT to function, as well as evaluate the important types of sonosensitisers available. The challenges associated with SDT will be discussed along with its potential solutions, through reviewing their past and current approaches.

Mechanisms in Sonodynamic Therapy

The primary way that SDT leads to cell death is the production of ROS when a sonosensitiser is ‘activated’ as a result of microbubble cavitation, which occurs when sound waves at a certain frequency range permeate through aqueous environments.[21] The first step in the mechanism involves the cavitation of microbubbles. Microbubbles are microscopic air/oxygen bubbles ranging from 1-100 μm, which have great potential in site-specific drug delivery.[22] These microbubbles already exist in aqueous environments, but can be deliberately administered intravenously to patients to increase the efficacy of SDT.[23] Under US exposure, they undergo cyclic expansion and contraction in a process known as cavitation, which induces rapid temperature and pressure changes. Cavitation can either be ‘stable’ or ‘inertial’, where microbubbles experience violent, uncontrolled oscillations at higher acoustic pressures.[24]

Inertial cavitation can subsequently cause sonosensitisers to rise to a higher energy state, leading to cytotoxicity through two possible mechanisms: sonoluminescence and pyrolysis. Sonoluminescence occurs when collapsing microbubbles release energy in the form of visible light.[25] Since most sonosensitisers are derived from photosensitisers, they are sensitive to light and will shift from their ground state to an excited state with higher energy. As they return to the ground state, they release energy to surrounding oxygen molecules to produce ROS, such as singlet oxygen, superoxide and hydrogen peroxide, which can damage intracellular DNA, promote lipid peroxidation, and result in apoptosis to targeted tumour cells, as shown in **Figure 1**.[26] Pyrolysis is similar in many ways; the only difference is that microbubbles shift sonosensitisers to an excited state by generating extreme amounts of

thermal energy, rather than light. It is believed that inertial cavitation produces shockwave effects which thermally dissociate water vapour into hydroxyl radicals and hydrogen radicals even without the presence of a sonosensitiser, which leads to cell death.[27]

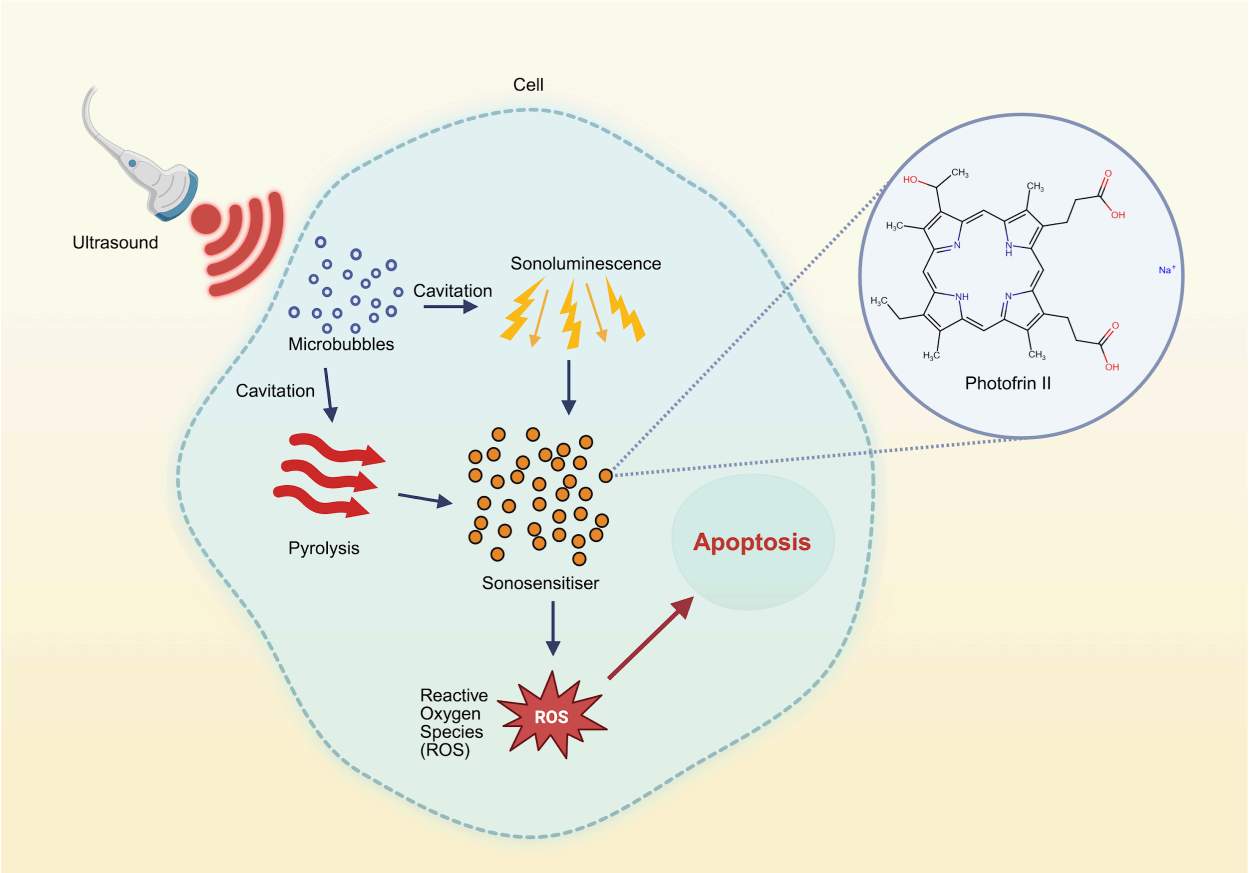

Figure 1: Schematic diagram to show microbubble cavitation leading to the generation of reactive oxygen species through sonoluminescence and pyrolysis, with Photofrin II as an example of a sonosensitiser. Figure created using https://BioRender.com and https://chemaxon.com/.

Microbubbles have a greater role than cavitation alone; they can enhance BBB permeability to aid drug entry into tumours and increase tumour specificity. This is especially useful in GBM, where the presence of the BBB would otherwise prevent certain drugs from entering. The BBB is a highly selective barrier formed by endothelial cells surrounding the brain's capillaries. It controls which substances can pass from the blood into the brain, shielding the central nervous system from toxins, pathogens, and chemicals.[28] In fact, the BBB blocks all large molecules and 98% of micro-molecule drugs from entering the tumour.[29] However, microbubble cavitation can cause significant shear stress on tumour cells and create ruptures in cell membranes in a process called cell lysis, thus enhancing the permeability of the tumour for sonosensitisers.[30,31] Another possible way microbubbles enhance permeability in tumour cells is through the generation of ROS, as mentioned earlier. Even without a sonosensitiser, microbubbles can generate ROS when in contact with US through pyrolysis, which is another possible explanation for increased tumour permeability.[32]

Furthermore, microbubbles can improve targeted drug release. Glioma tumours are rich in vascular endothelial growth factor (VEGF). Fan et al. (2013) designed VEGF-conjugated BCNU-loaded microbubbles, which specifically bind to VEGF receptor two overexpressed in GBM tumours, thus allowing localised drug release at the tumour site and reducing damage to healthy tissue.[33] This indicates

that microbubbles further increase the efficacy of SDT by targeted treatment and increased permeability in the tumour. Sonosensitisers are another main component in SDT, and the following section presents both organic and inorganic sensitisers, as well as their advantages and drawbacks.

Organic Sonosensitisers

Sonosensitisers have a greater ability to selectively accumulate in tumour sites due to the enhanced permeability and retention (EPR) effect in tumours.[14] When Yumita et al. (1989) found that several HPDs used in PDT were sensitive to US, they became the main focal point in sonosensitiser development, with Photofrin II and ATX-70 inducing severe cell damage when activated by US.[34,35] Organic sonosensitisers are mainly composed of carbon, particularly with carbon-hydrogen and carbon-carbon bonds. Despite having relatively good tumour specificity and ROS generation ability, even these commonly researched organic sonosensitisers have their downsides.

Photofrin II

Photofrin II is a family of oligomers where multiple hematoporphyrin molecules are bonded together, with the ability to retain in tumours longer than in healthy tissue selectively and has been FDA-approved as a photosensitiser in cancer PDT.[36] A study on the effects of Photofrin II compared the survival rates of leukemic (MT-2) and normal peripheral mononuclear cells (PMNC). Using ultrasound (450 kHz, 0.3-0.5 W/cm^2), MT-2 cell survival decreased with higher sensitiser concentrations, while healthy cells showed no difference in survival rate between groups with and without Photofrin II administration. In patients with acute-type adult T cell leukaemia, PMNC survival decreased from 69.4% ± 22.5% with ultrasound alone to 30.0% ± 23.0% with Photofrin.[37] Although survival rates of leukemic cells are still relatively high, Photofrin II dropped leukemic cell survival rates by half. At the same time, healthy tissue showed no difference in survival rates, suggesting that Photofrin II has minimal toxicity to healthy cells. However, since porphyrins are photosensitive, they may cause post-administration side effects, including skin sensitivity to light and external burns, as shown in **Table 1.** Furthermore, their low water solubilities call for the use of nanocarriers to enhance accumulation in tumour tissues.[38]

ATX-70

ATX-70 is a gallium porphyrin complex which showed the highest accumulation concentration in tumours out of the HPDs tested in this study by Nakajima et al. (1990). It also exhibits the longest phosphorescence lifetime among HPDs.[39] In other words, ATX-70 absorbs and reemits sonoluminescent light the longest while also selectively accumulating in tumours. The efficacy of ATX-70 was studied using isolated sarcoma 180 cells, and findings demonstrated that an 80 μM concentration of ATX-70 increased the rate of damage to the mice sarcoma by four times under 2 MHz US, compared to the two times increased rate with hematoporphyrin.[40] This suggests that ATX-70 significantly amplifies US-induced cytotoxic effects, primarily through the generation of singlet oxygen radicals, and potentially has a greater effect than Photofrin II and other HPDs. The in vivo effects of ATX-70 in Sprague-Dawley rats with mammary tumours were examined and showed that peak accumulation of ATX-70 in tumours was 24 hours post-administration, similar to Photofrin II.[41] 2 weeks after administration of ATX-70 and US radiation (3 W/cm^2), there was a mean reduction of tumour volume of 50%. With radiation of 5 W/cm^2, a further 18% reduction in tumour volume was observed compared to the control group (no treatment). ATX–70–mediated sonodynamic therapy significantly enhanced tumour regression and confirmed its efficacy as a sonosensitiser. Nevertheless, ATX-70 is a porphyrin derivative and shares the same downsides as Photofrin II, such as photosensitivity and low water solubility. Yumita et al. (2000) tested another HPD, known as ATX-S10, and found similar results to ATX-70 and Photofrin II, suggesting that HPDs share common characteristics and effects as a sonosensitiser.[42]

Rose Bengal

Another organic compound, Rose Bengal (RB), a xanthene dye, has also been tested for its potential cytotoxic effects when exposed to US. Umemura et al. (1999) have shown that RB increased ultrasound-induced cell damage to isolated sarcoma 180 cells by a factor of 2-3 at a concentration of 160 μM.[43] At a US exposure of 5.9 W/cm², cell viability dropped to as low as 4% in the presence of 160 μM RB, but only to 38% with US alone after 60 seconds. However, after comparing the results of this study to results of previous studies, it was observed that to achieve similar cytotoxic effects, RB concentration needed to be at least double the concentration of porphyrins, suggesting that porphyrins have a slight advantage in efficacy in terms of cell damage.[21,34,40] Another drawback of xanthene dyes is that the liver can easily capture and break them down, making them unable to accumulate in tumours.[44]

Inorganic Sonosensitisers

Titanium dioxide (TiO_2) nanoparticles

Inorganic sonosensitisers are substances that are not primarily composed of carbon and hydrogen in a chain. They fall into two categories: metal and non-metal. Titanium dioxide (TiO_2) nanoparticles (NPs) are a class of metal-based sonosensitisers that also produce ROS when activated by ultrasound.[45] As a superconducting material, it can produce electron-hole pairs when excited by ultrasound.[46] These pairs interact with nearby oxygen and water molecules to produce hydroxyl radicals and superoxide anions. An in vitro study suggested that TiO_2 NPs or US alone had little effect on mouse melanoma C32 cells, with cell viability remaining above 92% after US exposure at intensities up to 1.0 W/cm^2 for 10 seconds. However, combining a TiO_2 solution (0.500% w/w) with US at 1.0 W/cm² for 10 seconds resulted in a decrease in cell viability to 53.6 ± 1.8%, and apoptotic cell levels were 2.73 times higher than those in the control group (no treatment). Additionally, microscopic analysis confirmed that the TiO_2 particles were responsible for cell membrane damage, indicating the production of ROS.[47]

Despite its potential apoptosis-inducing ability, TiO_2 NPs tend to aggregate in physiological environments, which enables them to be easily captured by the reticuloendothelial system.[45] Therefore, research on carrier systems that can improve the dispersion stability of TiO_2 NPs under physiological pH conditions will significantly enhance the efficacy of TiO_2 NPs as sonosensitisers in SDT. You et al. (2016) encapsulated TiO_2 NPs in carboxymethyl dextran (CMD). This long-circulating hydrophilic TiO_2 NP still retained the ability to produce ROS when activated by US, but improved systemic circulation and uptake into cells.[48] Another study used an encapsulation of TiO_2 NPs in polyion complex micelles to improve their dispersion stability while producing singlet oxygen.[49]

TiO_2 NPs possess another drawback, namely, electron-hole recombination: As mentioned earlier, TiO_2 produces electrons and holes upon exposure to ultrasound. However, electrons and holes can recombine quite easily, resulting in reduced ROS production.[46] Other metal-based sonosensitisers that include Manganese-based composites and Fe_3O_4-loaded sensitisers also show antitumour effects, but may be limited in the complexity of their design and their reduced ROS production rate under non-acidic and hypoxic conditions. Therefore, more research should be done on overcoming these obstacles.[45]

Silicon nanostructures

Non-metal sonosensitisers have also been widely used in SDT research. Silicon nanostructures, for instance, can cause amplified sonodynamic effect, making them a potential sonosensitiser. A study found that silicon nanoplatforms (SiNPs) had a significant cytotoxic effect when paired with US: While US (0.88 MHz, 0.05 W/cm^2) alone only led to 50% Hep-2 cancer cell death in vitro, when combined with SiNPs (0.2 mg/ml), 90% of the cancer cells were destroyed.[50] The researchers also conducted in vivo experiments on mice with Lewis lung carcinoma (LLC). The mice received injections of a SiNP suspension (1 mg/ml) into the tumour. When exposed to US, tumour growth was inhibited by 30 ± 5% over the course of 13 days, further suggesting that SiNP may be a possible sonosensitiser in SDT. Furthermore, Mesoporous silica nanoparticles (MSNs) have been modified in the past to alter their hydrophilicity, enabling microbubbles to

be trapped in a hydrophobic MSN and increasing ROS production.[45] However, despite showing some promise in vitro and in vivo, the efficacy and safety of SiNP in human clinical trials remain unclear.[51]

Type	Sonosensitiser	Advantages	Disadvantages
Organic	Photofrin II	• FDA-approved for PDT.[36] • Significant tumour growth inhibition in vivo.[35,37] • Good accumulation in tumours.[36]	• High phototoxicity.[38] • Low water solubility.[38]
Organic	ATX-70	• Long phosphorescence lifetime.[39] • Significant tumour growth inhibition in vivo.[39,41,52,53] • Good accumulation in tumours.[39]	• High phototoxicity.[38] • Low water solubility.[38]
Organic	Rose Bengal (RB)	• Good tumour growth inhibition.[43]	• Requires higher concentration than porphyrins.[20,34,40] • Easily captured by the liver.[44] • Poor accumulation in tumours.[44]
Inorganic	Titanium dioxide nanoparticles (TiO_2 NPs)	• Good tumour growth inhibition.[45–47] • Can be modified to improve circulation and ROS production.[48,49]	• Aggregates and can be captures by the reticuloendothelial system.[45,48,49] • Electron-hole recombination reduces ROS production.[46]
Inorganic	Silicon nanoparticles (SiNPs)	• Medium tumour growth inhibition.[50] • Hydrophobic SiNPs can carry microbubbles to increase ROS production.[45]	• Antitumour effects are not as strong as those of porphyrins.[50]

Table 1: Advantages and disadvantages of inorganic and organic sonosensitisers.

Sonodynamic Therapy and Glioblastoma

In vitro experiments have demonstrated the potential of SDT in combating GBM. 5-Aminolevulinic acid (5-ALA), a precursor of porphyrins, has been extensively researched for its application in treating GBM. 5-ALA, at concentrations of 10 μm/mL, coupled with US at a power of 6 W, significantly reduced cell viability in rat RG2 glioma cells in vitro.[54] Similar observations were found in mouse cells in vitro as well as human U87 GBM cells.[55,56] Other sonosensitisers, including RB, sinoporphyrin sodium and PpIX have

also shown apoptotic effects in glioma cells.[57–59] Though SDT may not be a likely primary treatment in the future, it has high potential to become a supporting treatment. To date, research has shown that SDT can be combined with other therapies such as chemotherapy and PDT to yield synergistic effects.

Chemotherapy, as mentioned earlier, is a mainstream therapy used in GBM treatment. In the case of brain tumours, SDT can aid chemotherapy in a few ways. A major challenge associated with brain tumours is the presence of the BBB, which restricts molecules from entering the tumour site.[60] As mentioned earlier, cavitation of microbubbles can facilitate the entry of sonosensitisers into tumours.[61] By improving the selective uptake of chemotherapeutic drugs in cancer cells, SDT thereby reduces toxicity in normal cells and tissues.[62] SDT also enhances the sensitivity of cancer cells to chemotherapeutic drugs by inducing apoptosis and inhibiting ATP-binding cassette (ABC) transporters, including ABCG2. These transporters actively efflux chemotherapeutics, which would normally lead to tumour resistance to chemotherapy. However, SDT disrupts mitochondrial function and activates the mitochondria-caspase apoptotic pathway, both of which contribute to reduced ABC transporter expression.[63] Hence, the role of US and sonosensitisers can theoretically be used alongside chemotherapy to enhance treatment efficacy. When Wang et al. (2015) investigated the potential synergistic cytotoxic effects of PpIX-mediated SDT combined with the chemotherapeutic drug doxorubicin (DOX), their findings demonstrated that SDT significantly enhanced the efficacy of DOX by inducing apoptosis and increasing intracellular drug uptake.[62] When SDT was coupled with DOX, the ABC transporter P-glycoprotein (P-gp) was inhibited by 21.3%, leading to an increased uptake of the drug in K562/DOX cells, a multi-drug-resistant human leukaemia cell line. While DOX alone caused apoptosis rates of 7.9%, and US and PpIX caused apoptosis rates of 13.9%, Wang et al. (2015) found that together, SDT and chemotherapy caused the highest apoptosis rate of 39.6%, suggesting the synergistic effects of SDT and chemotherapy.

SDT stems from PDT, and along with it, sonophotodynamic therapy (SPDT) emerged with the combination of SDT and PDT. PDT, however, relies on light, which, by nature, is not penetrative, while SDT relies on US waves, which can be passed through the body through water molecules. US waves propagate through water molecules through a series of compressions and rarefactions. Therefore, activating sonosensitisers with a combination of US and light allows targeting tumours at different depths.[65] photosensitisers can be excited by using light with a certain wavelength, much like sonosensitisers with sound.[66] By activating sensitisers using both light and ultrasound simultaneously, SDPT exploits the synergistic effects of the two therapies to produce mechanical, sonochemical and photochemical activities, leading to apoptosis. Liu et al. (2016) conducted both in vitro and in vivo studies on the benefits of SPDT in human breast cancer cells and mouse mammary cancer cell lines, and found that SPDT was more effective than SDT or PDT alone.[67] In particular, while SDT alone caused a loss of cell viability of 27.36%-34.88% and PDT resulted in a loss of 36.69%-40.16% in vitro, the SPDT group exhibited a loss of cell viability ranging from 80.49% to 85.01%. Further analysis revealed a 12.68% survival rate for SPDT-treated cancer cells, which is substantially lower than that of SDT and PDT (68.78% and 59.51%, respectively). The evident synergistic effects of SPDT can be attributed to the increased generation of ROS. Specifically, the SPDT group produced intracellular ROS in mice 4-5 times more than SDT or PDT alone, suggesting that the heightened levels of ROS caused apoptosis in SPDT.

SPDT's effects on squamous cell carcinoma (SCC) tumour models were investigated.[68] While it was found that PDT alone resulted in a 40% reduction in tumour volume and PDT combined with TiO_2 sensitisers achieved 55% growth inhibition, the SPDT group exhibited an 80% tumour suppression. Increased ROS generation caused this increased effectiveness; however, more electron spin resonance (ESR) measurements showed that specifically hydroxyl radicals were most prevalent in SPDT tumours, being three times higher than in SDT or PDT alone. The results of these studies indicate a synergistic effect when SDT is combined with PDT. Though PDT and SDT alone may have mild effects, when combined together, SPDT has the ability to treat deep-lying tumours that light would not reach, such as bowel and ovarian cancer, or metastatic cancer that spreads to bone, lung and liver tissue.[69] SPDT is still a newly emerging combination, and its effects have still not been optimised in GBM treatment. A study found that in C6 rat models, its effects were initially promising, but its long-term impacts were less

effective than PDT alone.[70] SDT surprisingly contributed to tumour growth, and a possible reason for this was that the US was incorrectly applied. Although the effects of SPDT were small, ROS production in the SPDT group was far greater than in the SDT or PDT group.[70] Further research into sustaining SPDT's effects will be crucial for putting it into use in clinical settings.

Current Limitations and Insights of Sonodynamic Therapy

Sonosensitisers are a critical component of SDT, yet their development is still in progress. Due to a range of issues, including low stability, poor bioavailability, and selectivity, the current generation of sonosensitisers requires further research before SDT can become a more widely used treatment.[20] Some organic sonosensitisers, such as RB, for instance, have the ability to be triggered by US but are limited in their tumour accumulation ability. The lack of accumulation ability in some sensitisers has inspired research into the synthesis of their derivatives and investigations of their properties. For example, Sugita et al. (2007) investigated the development of tumour-accumulating derivatives of RB for SDT and PDT to overcome RB's poor tumour accumulation.[71] Several RB derivatives (RBD) were tested, including RBD1 (2, RB, C-2' alkyl ester, C-6 sodium salt), RBD2 (3, RB, C-2′ ω-carboxyalkyl ester, C-6 molecular form) and RBD3 (4, RB, C-2′ R-carboxyalkyl ester, C-6 molecular form). While all three derivatives behaved similarly to other sonosensitisers, RBD3 with longer alkyl chains significantly improved tumour accumulation, accumulating 1.5 times more in tumours than ATX-70 and 40 times more than RB. The researchers successfully engineered tumour-targeting RBDs by optimising amphiphilicity and showed that these modifications make RBDs promising sonosensitisers for SDT.[71] To further combat the low selectivity of sonosensitisers, Xiong et al. (2015) isolated a novel porphyrin derivative from Photofrin known as sinoporphyrin sodium (DVDMS), which showed strong potential in SDT due to its water solubility.[72] The results of this study demonstrated that DVDMS concentrations in tumours in vivo reached approximately 98.77% and 70.37% of the maximum concentration in the tumour at 6 h and 24 h, respectively. In S180 xenografted mice, DVDMS-mediated SDT also significantly decreased tumour growth rates;[72] It outperforms other porphyrin-based sonosensitisers due to its superior tumour selectivity, effective ROS generation and strong tumour inhibition, deeming it a promising solution, particularly when compared to the current sonosensitisers and their individual disadvantages.

The role of microbubbles can also be expanded in SDT. As mentioned earlier, microbubbles facilitate the activation of sonosensitisers through cavitation and pyrolysis. A promising potential of microbubbles was investigated by Nimokou et al. (2012), when they covalently attached RB to a lipid microbubble to form a conjugate, which showed heightened cytotoxicity in tumour cells and a greater reduction in tumour growth compared to the use of RB alone.[73] First, microbubble conjugation provides site-specific, precise drug delivery, as US selectively collapses microbubbles, activating only sonosensitisers around the tumour site. Second, the close spatial proximity of RB to the microbubble enhances the effectiveness of ROS production. In fact, when the cytotoxic potential of the MB-RB conjugate was assessed, it was found that exposure to US (1 MHz, 1.5 W/cm^2 for 30 s) decreased cell viability by 72% in RIF-1 murine fibrosarcoma cells in vitro.[73] The results also indicated that the MB-RB conjugate had a significantly enhanced production of singlet oxygen compared to unconjugated RB, supporting the potential of MB-RB conjugates as a highly effective approach in SDT. Furthermore, microbubbles can be used to identify tumour locations more accurately: Zheng et al. (2012) developed hematoporphyrin-encapsulated poly(lactic-co-glycolic acid) microbubbles, which acted as a contrast agent for ultrasound imaging both in vitro and in vivo.[74] This suggests that microbubbles may have a greater use, not only in SDT delivery, but also in cancer diagnosis.

Another possible function of microbubbles in SDT is to combat hypoxia levels usually associated with tumours. A compromised and anisotropic blood supply in tumours leads to inadequate oxygen and nutrient delivery to tumour cells.[75] As a result, SDT, which relies on oxygen acting as a substrate for the

generation of ROS, becomes less effective and limited in its cytotoxic potential. To combat hypoxic environments, oxygen-loaded MBs (OxyMBs) conjugated with RB (OxyMB-RB) were investigated.[23] A series of in vitro and in vivo experiments was conducted. However, notable findings in the in vivo experiments revealed that OxyMB-RBs resulted in a 45% reduction in tumour volume over 5 days with US (1 MHz, 3.5 W/cm^2, 30% duty cycle, 3.5-minute exposure). In contrast, a non-oxygen-loaded MB conjugate (SF6MB-RB) resulted in a 35% increase in tumour volume. The significant difference in tumour growth between OxyMB-RB + ultrasound and SF6MB-RB + ultrasound indicates that the oxygen delivery is crucial for SDT efficacy in hypoxic tumours.[23]

As stated earlier, the cavitation effect of microbubbles can temporarily open up the BBB and improve drug uptake by tumours in the brain. However, the ability of US to open the BBB also poses risks. Excessive exposure of microbubbles to focused US can significantly reduce the energy threshold for brain lesion creation, causing vascular injury and haemorrhage. [76] Compromising the BBB could allow cells to escape the tumour and may lead to an increased risk of metastasis. However, under a certain threshold, there is the possibility of a 100% BBB opening rate without any haemorrhage or vascular damage.[77] More research and in vivo studies are needed to verify the effects of microbubble cavitation on the BBB. To mitigate the possibility of rupture and haemorrhage, O'Reilly and Hynynen (2012) tested a real-time feedback-controlled focused US system which monitors inertial cavitation in the body and adjusts the ultrasound pressure according to the feedback data. They found that this system could be a reliable way to prevent excessive exposure to US, ensuring the BBB remains open temporarily with minimal rupture and vascular damage.[78]

In both clinical applications and future studies, providing real-time monitoring of treatment parameters, such as ROS concentration, is crucial. Extreme concentrations of ROS generated may lead to temperatures too high, and can result in damage to surrounding healthy tissue; therefore, more research on optimising and standardising SDT parameters is beneficial.[20] A proposed insight into quantitatively measuring ROS concentration is through chemiluminescence, where probes detect the emission of light from chemical reactions occurring in SDT. Several researchers have developed chemiluminescence probes: Hu et al. (2011) developed a near-infrared area charge-coupled device, which detected chemiluminescence in 2-dimensional imaging in vivo, showing a linear relationship between singlet oxygen concentration and chemiluminescence, making quantification of ROS concentration possible in vivo.[79] Zhen et al. (2016) engineered chemiluminescent semiconducting polymer nanoparticles which were sensitive to chemiluminescent irradiation by hydrogen peroxide, and they were able to detect concentrations as low as 5 nM in vivo mouse models.[80]

Conclusion

SDT has been developed to treat various cancers, including GBM. SDT starts by inducing microbubble cavitation through US, leading to ROS generation from sonosensitisers. ROS is responsible for cell damage and apoptosis of tumours. Numerous sonosensitisers have been researched and tested. Currently, Photofrin II, an organic sonosensitiser, is being used in clinical applications for PDT. While it shows good antitumour effects, it is limited in its low water solubility and side effects on the patient. Inorganic sonosensitisers, however, show weaker antitumour effects but can be more easily modified to increase their efficacy. SDT has a high potential in treating GBM due to its ability to open up the BBB for a higher accumulation in tumours, especially when combined as an adjunctive treatment to other therapies, such as chemotherapy or PDT. However, more research and in vivo experimentation are needed to optimise this newly emerging therapy; it is limited by current sonosensitiser development and a lack of real-time monitoring of parameters, such as ROS concentration. Recent studies have revealed that microbubbles may have a greater role in optimising SDT since they can be conjugated to sonosensitisers to improve tumour accumulation or combat hypoxia conditions, which decrease the efficacy of SDT. Furthermore, utilising chemiluminescent probes in real time may improve the effectiveness of SDT in clinical applications, as well as enabling future studies to quantify ROS concentrations. SDT, however, will only be used in clinical applications through more experimentation and collaboration among researchers.

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