Chapter

Sonodynamic and Photodynamics Used as a Combined Therapy in the Treatment of Malignant Neoplasms: Facts and Open Questions

Heber Lopes de Mello, Luiz Anastacio Alves, Evellyn Araujo Dias, Sabrina de Sá Pereira Magalhães, Vinicius Cotta-de-Almeida and Rodrigo da Cunha Bisaggio

Abstract

Photodynamic therapy (PDT) used in combination with sonodynamic therapy (SDT) is a new approach that aims to increase the effectiveness of tumor treatment when compared to the effect of each independent therapy. PDT is based on stimulating sensitizers with photons, while the most accepted theory for SDT is that sensitizers are stimulated by the sonoluminescence phenomenon. However, after the excitation of the sensitizer, both therapies follow a common path, leading to the generation of free radicals and inducing cell death. One of the positive aspects of this combination is the augmentation of anti-tumor activity with fewer side effects, since cell death may be induced using lower sensitizer concentrations or less exposure to ultrasound or light. Another benefit of combining PDT and SDT, especially with the use of low-frequency ultrasound is the induction of sonophoresis. For instance, on the skin, it may facilitate the absorption of the sensitizer. However, research involving both PDT and SDT exhibit many variants, including differences in irradiation sources and their intensities, among others. These aspects contribute to a lack of standardization, leading to result variations, hindering assessment on the real contribution that these combined therapies can offer in tumor treatment. Thus, further research in the pre-clinical and clinical areas are crucial.

Keywords: cancer therapy, ultrasound, sonodynamic therapy, sonophotodynamic therapy, sonophoresis

1. Introduction

Currently, medicine has gone through great advances due to basic and applied research, as well as the implantation and discovery of new technologies. However, the treatment of malignant neoplasms (cancers) requires improvement. According to the WHO, malignant neoplasms were the second leading cause of death worldwide in 2018 [1], and CDC estimates indicate that malignant neoplasms will be the main cause of death in the USA by 2030 [2].

Malignant neoplasms are a group of diseases exhibiting the common characteristic of invasion of adjacent tissues by proximity or migration to other tissues and organs by lymphatic, blood circulation or body cavities, in a process known as metastasis. Malignant neoplasms exhibit six basic characteristics defined by Hannah and Weingberg in 2000: they are able to resist cell death, induce angiogenesis, exhibit replicative immortality, evade growth suppression, activate invasion (metastasis) and sustain proliferative signaling [3]. The origin of malignant neoplasms is still unknown, and several theories have been postulated in this regard, including Somatic Mutation theory, Evolutionary theory and Cancer Stem cell theory [4, 5]. Environmental factors, such as ultraviolet radiation, ionizing radiation and carcinogens, may alter the genetic structure of cells and explain a portion of cancer cases. To illustrate oncogenesis complexity, smoking is known to increase the risk for cancer up to 100-fold. However, when comparing the mutation numbers of lung cell carcinomas in smokers and non-smokers, this increase is only 1.15-fold [4]. Today, it is clear that the tumoral microenvironment is also part of the oncogenesis process.

Traditional cancer treatments, such as chemotherapy, radiotherapy and surgery, in spite of being effective for several types of tumors, saving millions of lives, are often aggressive, expensive and not always efficient. Chemotherapy is based on the use of drugs in order to reach and destroy tumor cells and radiotherapy applies ionizing radiation to destroy and prevent tumor growth. These therapies are the most common in cancer treatments, but they affect not only tumor cells but also healthy ones, which can lead to side effects, such as nausea and hair loss. Surgery can be used in several cases in which the tumor can be removed either partially or completely, although it can be very invasive and expensive [6]. These treatments are often combined to enhance results, increasing the possibility of longer remission times and, in some cases, cures. Other treatment modalities have begun to be recently applied, such as Immunotherapy, Hormone Therapy and Stem Cell Transplant, alongside, or not, traditional treatments.

Photodynamic therapy (PDT) and sonodynamic therapy (SDT) emerge as alternative or adjuvant treatments for cancer cases, exhibiting a minimally invasive approach. These therapies are based on the administration (either systemic or topical) of a photosensitizer (PS) or a sonosensitizer (SS), generally non-toxic when used in the appropriate concentrations, resulting in cell death when irradiated with light or ultrasound [7].

2. Ultrasound applied to medicine

2.1 Ultrasound in biological systems

Ultrasound is a mechanical wave exhibiting frequencies above 20 kHz, out of the human hearing range [8]. Historical evidence indicates that ultrasounds were first applied in humans to examine a brain tumor by Karl Dussik, in 1942 [9], who reported that, when ultrasound is focused, its biological effects are more localized [10]. Ultrasound applied to therapeutic purposes can be used at low intensities (0.125–3 W/cm^2) to stimulate normal physiological responses to injury and facilitate the transport of substances across the skin (sonophoresis), or it can be used at high intensities (>5 W/cm^2) to selectively destroy target tissues [11].

Acoustic cavitation occurs when ultrasound waves pass through an aqueous medium and it is an important ultrasound interaction with biological tissues. The disturbance caused by ultrasound causes oscillations in the ambient pressure that can lead to gases present in the solution to form small “bubbles”. With the maintenance of the ultrasound waves, these bubbles may continue to increase in size and, eventually,

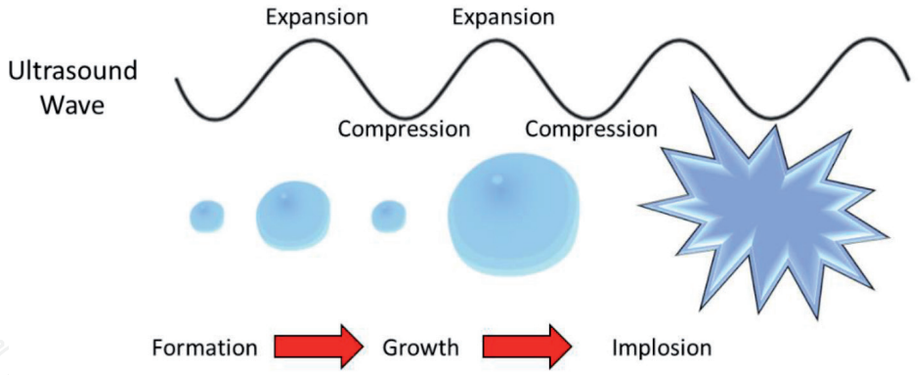

Figure 1.
Scheme depicturing the development of cavitation bubbles. The ultrasound waves cause the cavitation bubble to increase and decrease in size in sustained cycles, until the bubbles reach an unstable and critical size. When the bubbles implode, high temperatures and the release of energy in the form of light (Sonoluminescence) can occur.

collapse (**Figure 1**), releasing energy [12]. Another ultrasound effect is thermal; where the mechanical waves cause frictional heat in tissues by molecule vibration and, depending on the temperature, lead to different biological effects [8]. Many factors can influence US treatment outcomes, such as ultrasound exposure duration, heated tissue volume, maximum temperature achieved and rate of temperature increases [11]. This effect is routinely applied in the physiotherapy area to treat osteo-muscular lesions.

2.2 Ultrasound in malignant neoplasm therapy

The first clinical application of ultrasound to malignant neoplasm was performed in 1944 and, since then, several studies have been carried out applying this technique [13]. The consolidation of ultrasound to treat tumors originates in prostate neoplasm treatments that began in the 1990s, by the use of High-intensity focused ultrasound (HIFU), whose intensity applied to tumors may range from 100 to 10,000 W/cm^2 [14].

Almost at the same time, Yumita et al. discovered sonodynamic therapy (SDT), by associating hematoporphyrin derivatives (already applied in PDT) to ultrasound irradiation, observing cell damage up to 50% higher compared to the non-associated treatment [15]. Although it acts in a similar manner as PDT, the exact sonodynamic therapy activation mechanism of a certain molecule (sonosensitizer) is not as well elucidated as in photodynamic therapy. The two main hypotheses concerning SDT mechanisms have been postulated, both directly linked to acoustic cavitation (see above). The thermal effect hypothesis suggests that heat is released, leading to temperatures of up to 10,000 K, with pressures reaching 81 MPa after the collapse of cavitation bubbles, which would be responsible for sonosensitizer activation [16]. The second and currently widely accepted hypothesis is that sonosensitizer activation occurs due to an effect known as sonoluminescence, where light energy is released after the collapse of the cavitation microbubbles [17]. This light energy would be, therefore, responsible for sonosensitizer activation, which leads to the production of free radicals and, consequently, cell death (**Figure 2**) [18]. In addition to the use of low-intensity ultrasound as a treatment using SDT, another interesting feature is the possibility of facilitating the entry of molecules through the plasma membrane, as demonstrated by Harrison and Balcer-Kubiczek in the early 90s when irradiating Chinese hamster ovaries cells with low intensity ultrasound, favoring the entry of adriamycin and amphotericin B, evidenced by increased cell death [19]. Low frequency ultrasound (<100 kHz) is more efficient in increasing skin permeability

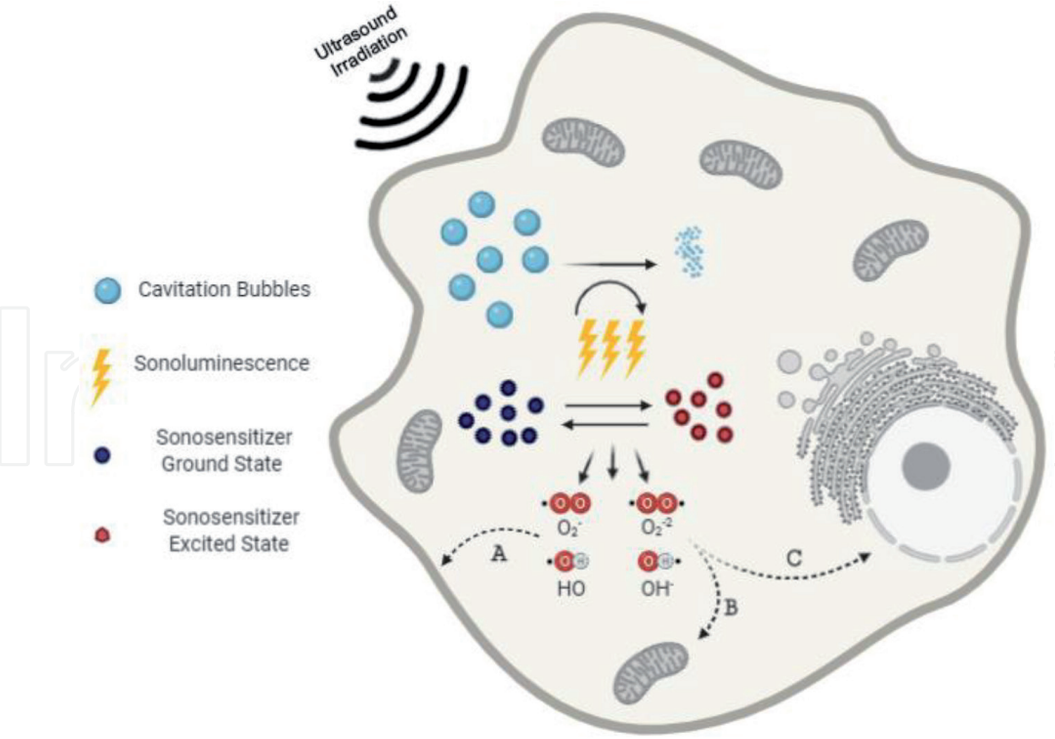

Figure 2.
Scheme representing the potential SDT mechanism. Ultrasound irradiation induces intracellular cavitation bubbles. The collapse of these bubbles, through sonoluminescence, generates an energy that will be responsible for the activation of the sonosensitizer from its fundamental state to an excited state. As the activated sensitizer returns to ground state, the released energy is then transferred to the oxygen present in the cytosol to produce high amounts of ROS, including oxygen peroxide and superoxide ions. These can cause several cell changes that may result in cell death, such as (A) oxidative degradation of lipids, that would damage cell membranes, organelles and vesicles that are made-up of lipids. (B) Damage to the mitochondrial membrane, initiating the apoptotic process mediated by cytochrome C. (C) Direct damage to structures and molecules essential for cellular homeostasis.

than therapeutic ultrasound. This is attributed to the cavitation phenomenon, which is more frequent at low frequencies [20]. Tachibana et al. identified that the blood glucose levels of hairless rats immersed in glasses containing an insulin solution (20 U/mL) and placed in an ultrasound bath (48 kHz) decreased by 50% in 240 minutes [21]. The use of ultrasound as a drug delivery mediator technique represents an important technological advance in many areas. This is no different in PDT treatment, as it may be possible to facilitate tissue molecule entry, reducing the amount of sensitizers to be used in treatments, in addition to allowing a joint sonodynamic and photodynamic therapy effect, since both therapies work in a similar manner.

3. Sono-photodynamic therapy (SPDT) used as a combined therapy

PDT is performed in two main stages. The first comprises patient photosensitizer administration followed by exposing the target region of the therapy to light at an appropriate wavelength to produce reactive oxygen species capable of causing the death of microorganisms or abnormal cells, such as tumors [22]. The wavelength in which the photosensitizer absorbs energy is an important aspect that must be taken into account when choosing the sensitizer. Capella and Capella identified molecules that absorb light between 600 and 800 nm as ideal since, below this range, hemoglobin, the main light-absorbing protein in the blood, would compete for the energy emitted to activate the photosensitizer and, above this range, photons would not have enough energy to participate in photochemical reactions. However, PDT exhibits a major limitation regarding tissue penetration [23]. Bashaktov et al. pointed

out that skin light penetration ranges from 1.5 mm to 2.5 mm [24], and Kondo et al. reported that light penetration may reach 7 mm in mucous membranes [25]. In the light of these facts, PDT becomes limited to surface region treatments or treatments for surfaces located close to the irradiating source, demanding invasive techniques in the case of internal tissue and organ treatments. In this scenario, sonodynamic therapy may be an alternative, due to the ability of ultrasound to penetrate deeper into the organism, reaching internal tissues and organs. As a result, the combined use of these therapies may be extremely positive. Sono-photodynamic therapy has been considered more effective than the individual therapies as a possible cancer treatment, as verified in a glioma model [26]. The idea is to use both light and sound to activate a sono-photosensitizer, leading to the destruction of tumor cells. Besides the action of US facilitating the sensitizer cell permeability, as described previously, another advantage of the combined use of these two therapies is the possibility of reducing the sensitizer dosage without reducing treatment effects [27], since most assessed sonosensitizers, i.e. porphyrin derivatives, are also photosensitizers [28]. Some *in vitro* studies have already attempted to prove the effectiveness of this therapy. For example, Li et al. studied the effects of SPDT on C6 glioma cells using hematoporphyrin monomethyl ether (HMME) as a sensitizer, by ultrasound and light irradiation, respectively. The C6 glioma cells treated with SPDT exhibited higher growth inhibition than cells treated by the individual therapies [29]. Furthermore, Zhu et al. used SPTD on HEPG2 cells, applying curcumin-loaded poly (L-lactide-co-glycolide) as a sensitizer [30]. Higher apoptosis percentages and better anti-cancer cell proliferation effects were observed in the group treated with SPDT compared to the groups submitted to either SDT or PDT. An *in vivo* study performed by Jin et al. tested the combined therapy on C3H/HeN mice, using ATX-70 and PH-1126 as sensitizers. The percentages of tumor inhibition observed in mice treated with ATX-70 were 92, 77 and 27% when submitted to SPDT, SDT and PDT respectively, and the group treated with the combined therapy exhibited an increase in 120-day survival rates to 60% in five mice, higher than in the SDT and PDT groups. When using PH-1126 as a sensitizer, tumor inhibition results of 98, 76, or 43% were observed for SPDT, SDT and PDT respectively, and the combination therapy increased 120-day survival rates to 88% in eight mice [27]. In addition, the combined therapy has also been tested in humans as summarized in **Table 1**. Li et al. evaluated seven patients with advanced gastric and esophageal adenocarcinoma who, after treatment with SPDT, showed improvement of the condition [31]. Wang et al. studied twelve patients with advanced breast carcinoma and, after treatment with SPDT, reported a median patient survival exceeding 14.5 months [32].

Number of patients	Treatment	Results	References
7	Sensitizers applied sublingually, followed by SPDT once a week	Three patients achieved a complete response, three, a partial response and one, an MR response	Li et al. [31]
12	Sensitizers applied sublingually, followed by SPDT once a week	Two patients achieved a complete response, seven, a partial response and three, a stable disease state	Wang et al. [32]
115	Sensitizers applied sublingually, followed by SPDT once a week	Of the 115 evaluated patients, 70 exceeded the calculated life expectancy	Kenyon et al. [33]

Table 1.
Summary of clinical research results involving SPDT.

Kenyon et al. in a study carried out with 115 patients followed for 4 years, reported a significant life expectancy increase for patients diagnosed with breast, bladder, colorectal, prostate and ovarian cancers, among others [33]. It is important to standardize Sono-Photodynamic therapy experiments according to the sensitizer, the exposure times and light and ultrasound intensities, so that the results may be compared, repeated and discussed worldwide, aiming at minimally invasive applications to neoplasm treatment with minimal patient discomfort.

4. Conclusions

PDT and SDT have great potential to be used as treatments for several diseases, especially cancer. However, when used together (SPDT), their effects can be even more significant. Nonetheless there are many experimental variables that interfere in the outcome of the treatment. This fact leads to difficulties in comparing the results of assays performed by different groups around the world, either *in vitro* or *in vivo*. In order to appraisal the real potential of SPDT, further studies must be carried out in a more standardized manner, controlling the irradiation times, intensities of irradiation by light and ultrasound, type of sensitizer used and even the type and stage of the tumor. Thus, further research in the pre-clinical and clinical areas are crucial. In the case of clinical studies, double blind randomized controlled trials of sonodynamic therapy alone and combined with photodynamic therapy are required.

Author details

Heber Lopes de Mello[1,2], Luiz Anastacio Alves[2]*, Evellyn Araujo Dias[2,3], Sabrina de Sá Pereira Magalhães[2,4], Vinicius Cotta-de-Almeida[1] and Rodrigo da Cunha Bisaggio[2,3]

1 Thymus Research Laboratory, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil

2 Laboratory of Cellular Communication, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil

3 Biotechnology Departament, Federal Institute of Education, Science and Technology of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

4 Biomedicine Department, Federal University of the State of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

*Address all correspondence to: alveslaa@gmail.com

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