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Abstract

Photodynamic therapy is a well-established and clinically approved treatment for several types of cancer. Antineoplastic photodynamic therapy is based on photosensitizers, i.e., drugs that absorb photons translating light energy into a chemical potential that damages tumor tissues. Despite the encouraging clinical results with the approved photosensitizers available today, the prolonged skin phototoxicity, poor selectivity for diseased tissues, hydrophobic nature, and extended retention in the host organism shown by these drugs have stimulated researchers to develop new formulations for photodynamic therapy. In this context, due to their amphiphilic characteristic (compatibility with both hydrophobic and hydrophilic substances), liposomes have proven to be suitable carriers for photosensitizers, improving the photophysical properties of the photosensitizers. Moreover, as nanostructured drug delivery systems, liposomes improve the efficiency and safety of antineoplastic photodynamic therapy, mainly by the classical phenomenon of extended permeation and retention. Therefore, the association of photosensitizers with liposomes has been extensively studied. In this review, both current knowledge and future perspectives on liposomal carriers for antineoplastic photodynamic therapy are critically discussed.

Key words: Photodynamic therapy; Liposome; Antineoplastic therapy; Cancer; Photosensitizer

Introduction

The cytotoxic effects of photodynamic therapy (PDT) on tumor tissues were first described at the beginning of the 20th century, but this therapy would be widely used in medical services only in the 1990s. Nowadays, PDT is a well-established antineoplastic therapy with several commercially available protocols approved for different types of cancer. It has proven to be a cost-effective treatment compared to other traditional therapies (1), and this may be due to the low costs of PDT equipment and hospital facilities in comparison to the requirements for other therapies such as surgery, chemotherapy and radiotherapy. All of these advantages have stimulated the emergence of several centers for PDT treatment worldwide, including emerging countries such as Brazil and China (1,2).

PDT is based on the delivery of photosensitizer drugs (PSs) to biological targets, which are then irradiated with light at specific wavelengths (3). There are many different kinds of PS, each one with its specific excitation wavelength. However, the useful spectral window for PDT application in biological tissues is limited by the absorption of light by both water - at wavelengths above 1000 nm - and hemoglobin/myoglobin - at wavelengths below 600 nm (4). Therefore, the excitation wavelength must be in the 600-1000-nm range so that the light can cross the cells without being absorbed before reaching the PS. During PS photoactivation, an excitable electron is promoted to a higher energy level (antibonding orbital) and the PS reaches the first excited singlet state. This excited state decays to a lower energy level by emitting fluorescence or, alternatively, it decays by the process of intersystem crossing to a state named triplet (3). This triplet state is long lived and, because of this feature, it is able to react with triplet oxygen molecules and other biomolecules or to emit phosphorescence (3). When it transfers energy to triplet oxygen, very reactive species called singlet oxygen are produced. Both the triplet PS and the singlet oxygen are unstable molecules and for this reason they are responsible, in biological tissues, for the damage to biomolecules - mainly polyunsaturated lipids,

nucleic acids and proteins - after PS irradiation. Didactically, the direct reaction between triplet PS and a biomolecule is called the Type 1 reaction and this process leads to PS blanching; the production of singlet oxygen by the triplet PS is called the Type 2 reaction and it regenerates the ground-state PS (4) (Figure 1).

PSs are classified as 1st, 2nd, and 3rd generation, or according to their respective chemical class (e.g., phenotiazine, porphyrins, chlorins, phthalocyanines) (3,5). Despite their effectiveness on tumor cells, the majority of these PSs have shown limitations to their use in clinical applications, which include extended retention in the host organism, substantial skin phototoxicity, low solubility in physiological solutions, low selective accumulation in the diseased tissue, and inappropriate half-lives within tissue, impairing

the optimization of illumination schedules (6).

Several approaches were tested in order to avoid the undesired effects described above and the employment of drug delivery systems has then emerged as a new strategy to improve the efficacy and safety of PDT. The drug delivery system is expected to incorporate high amounts of the PS without loss or alteration of its activity, to selectively accumulate the PS in the diseased tissue, to deliver therapeutic amounts of PS to the target site, to protect the PS from degradation and from premature clearance, to minimize drug leakage during transit to target, and to facilitate PS interaction with cells; moreover, the delivery system must be biodegradable and should induce little or no immunogenicity (7).

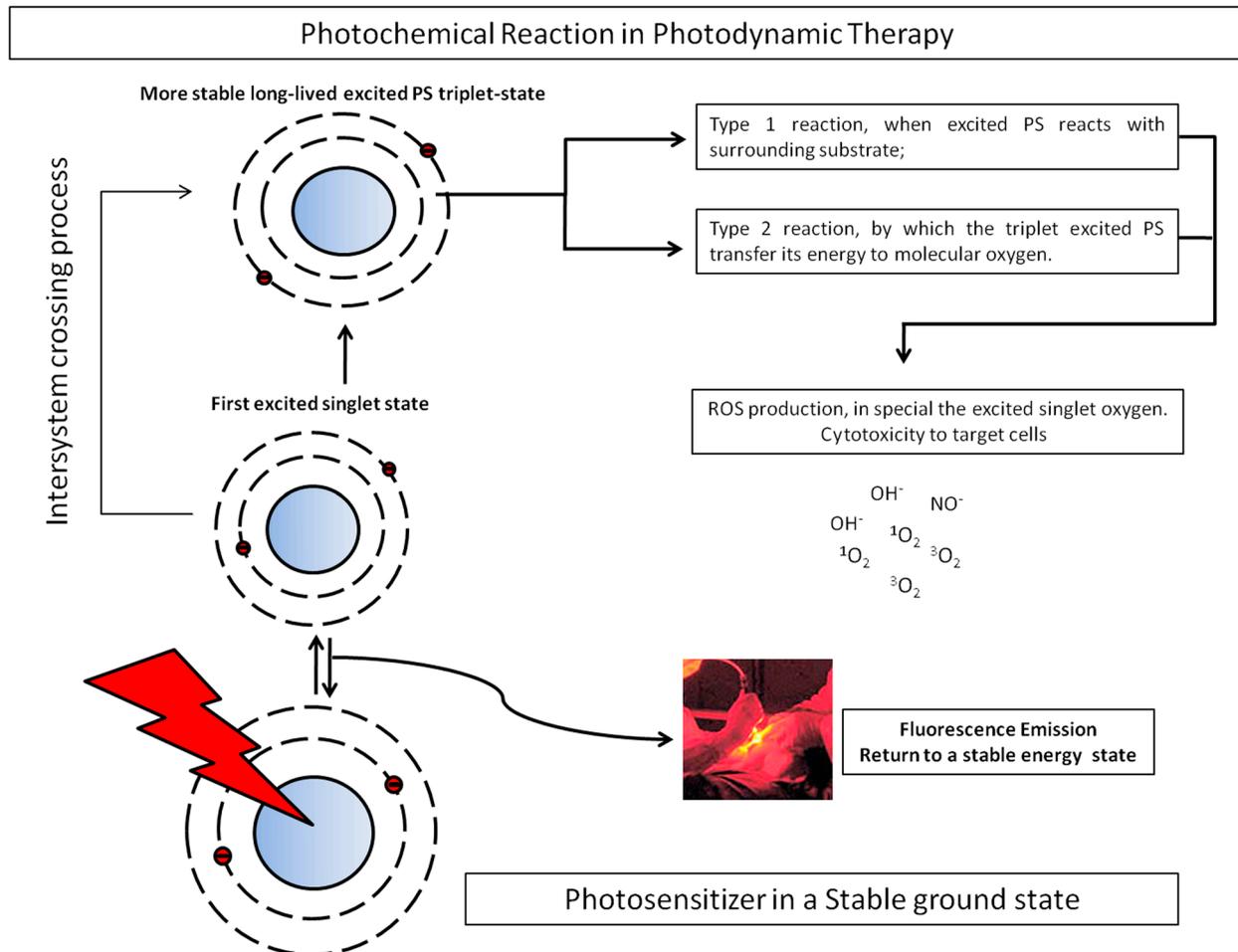In this context, it is not by chance that liposomes are

Figure 1. When excited by light at a specific wavelength, the photosensitizer (PS) reaches its first excited singlet state. This state can decay by emitting fluorescence or, alternatively, it reaches the more stable excited triplet state through the intersystem crossing process. The PS in its triplet state can directly react with biomolecules (Type 1 reaction) or with the triplet oxygen producing the singlet oxygen (Type 2 reaction), which is much more oxidative than the triplet oxygen. Although both of these reactions can damage cells, the most significant damage is induced by Type 2 reactions. On the right side is a photograph showing a patient being laser-irradiated: fluorescence emission can be used for detecting the photosensitizer.

one of the most extensively studied drug delivery systems and represent a significant proportion of nanotechnology research in biomedicine today (8). These lipid nanoparticles possess several features that are desired in a drug delivery system (9). Moreover, a large number of investigations on the subject of liposomes have provided some crucial improvements in liposomal formulations, such as: prolonged circulation time in the bloodstream (PEGylated liposomes), improved dose and target selectivity (immunoliposomes, tumor-specific ligands, pH-sensitive and magnetoliposomes) and enhanced cell internalization (cationic liposomes) (7). However, despite their advantages, some kinds of liposomes are not in current use inasmuch as none or few studies showing their application for PDT have been reported since their first description. Therefore, only those liposome approaches showing encouraging *in vivo* results for anticancer PDT are described in the present review. In addition, new perspectives on liposome-based antineoplastic PDT are discussed.

General aspects of liposomes

Liposomes are artificial vesicles generally ranging in size from 20 to 1000 nm (8), composed mainly of a phospholipid bilayer surrounding an aqueous core (see Figure 2). The phospholipids employed to produce liposomes can be synthetic or derived from natural sources. Their structure is characterized by 2 regions - polar head and hydrophobic long hydrocarbon acyl chains - which can be modified to suit the desired liposome size and shape (9).

The structures of both the hydrophobic and hydrophilic regions of a phospholipid determine its shape. For example, phospholipids such as phosphatidylcholine, phosphatidylserine and sphingomyelin are known to be cylindrical-shaped lipids because the diameter of their

polar group is similar to that of their hydrophobic part, causing them to spontaneously form bilayers when dispersed in an aqueous medium. On the other hand, phosphatidylethanolamine and cardiolipin are cone-shaped lipids because the diameter of their polar head is significantly different from that of their hydrophobic region - these lipids are also called non-bilayer lipids as they preferentially form a hexagonal structure (depending on the conditions of the medium) (9).

Since there is a wide variety of phospholipids, it is possible to change the liposome size, charge, and surface properties by adding new ingredients to the lipid mixture. Therefore, it is important to select phospholipid combinations and to standardize appropriate PS concentrations
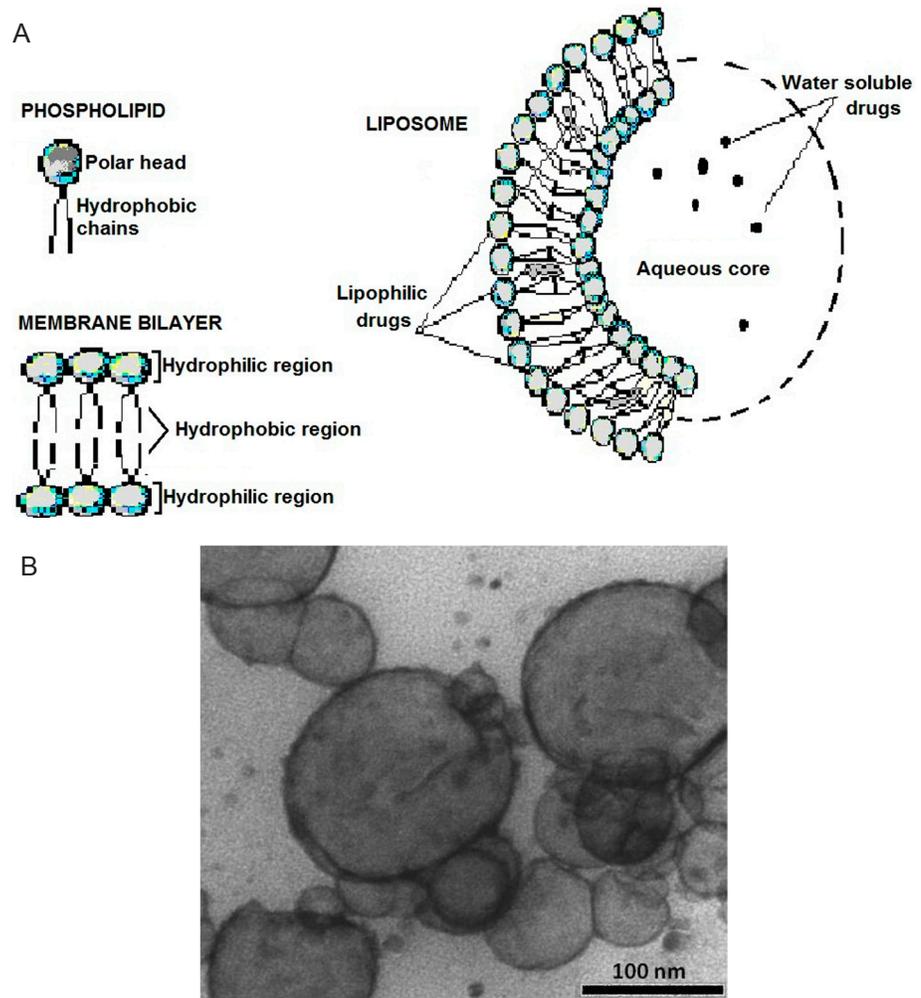
Figure 2. *A*, Liposomes are basically composed of a phospholipid bilayer surrounding an aqueous core; they function as containers in which several chemically different compounds can be entrapped or to which they can be attached. Hydrophobic drugs can be associated with the lipid membrane while the hydrophilic ones can be dissolved in the aqueous core. *B*, Transmission electron microscopy images of liposomes. The small black particles are artifacts produced during sample preparation for microscopy.

and entrapment methods in order to improve PS-loaded liposomes. In general, the methods frequently employed to encapsulate PSs are rehydration of a thin lipid film (classic Bangham technique), ethanol injection and freeze/thawing (10-12). It should also be taken into account that the chosen method - sometimes involving mechanical energy, chemical reactions or temperature variation steps - should neither affect PS properties nor reduce its activity.

The stability of the liposomes should also be evaluated since the association of PS with liposomes has been shown to alter the thermal phase behavior of the latter (10,13). This parameter is intrinsically dependent on the phospholipid-specific phase transition temperature at which the molecules are reorganized from a rigid bilayer to a fluid bilayer. It has been reported that the association of the PS temoporfin resulted in a concentration-dependent decrease in the phase transition temperature of dipalmitoylphosphatidylcholine-based liposomes (13). When the lipid composition was modified with phospholipids endowed with longer hydrophobic acyl chains (distearoylphosphatidylcholine), phase transitions were well above body temperature even at high PS load (13). Moreover, the lipid bilayer phase transition can also be modified with the addition of cholesterol, where increasing amounts of cholesterol increase liposome rigidity and stability in biologic fluids (13).

Photosensitizer liposome formulations

One of the first PSs used for cancer therapy consisted of a complex mixture of several partially unidentified porphyrins. Besides their effectiveness in PDT treatments, poor selectivity for diseased tissues, high dose, extended retention in the host organism, and low wavelength activation (630 nm) were the main drawbacks (7,14). These drawbacks stimulated the development of new PSs showing fast elimination from the body, fewer side effects, and a higher absorption peak (650-800 nm) (6,7,15).

Despite the improvements achieved, these molecules present low solubility in physiological solutions (6,7,15). For instance, both phthalocyanines and chlorins are hydrophobic PSs that form aggregates in physiological solutions (16). When aggregated, the PSs are far less efficient in translating light energy into a chemical potential. This happens because the effect of quenching is amplified in these aggregates, i.e., the aggregated molecule is not able to absorb light or, even though it absorbs light, the photoexcited PS decays to the ground-state before producing singlet oxygen (16). Moreover, PS aggregates also hamper parenteral administration (such as intravenous injection) and the delivery of the PS drugs to targeted tissues (6,7). One may think that the use of a hydrophilic PS should minimize the drawbacks shown by hydrophobic PSs, but the hydrophilicity raises problems related to the interaction with biological tissues. In fact, it has been reported that the hydrophilic photosensitizer aminolevulinic acid (ALA) is not well internalized by cells

and has no specificity for diseased tissues (6). Therefore, the use of PSs associated with nanoparticles could be an interesting approach in order to avoid PS aggregation and to deliver the PS into the target site.

In this context, liposomes are suitable delivery systems for carrying hydrophilic or hydrophobic PSs, improving their clinical application (17). The liposome structure enables the entrapment of hydrophilic PSs in the aqueous core and the attachment of the hydrophobic ones to the phospholipid bilayer; consequently, the interaction of hydrophilic PSs with cells is enhanced and the hydrophobic PSs are kept in their monomeric configuration even in physiological aqueous media. In general, hydrophobic PSs have been attached to small unilamellar vesicles (20 to 100 nm) - whereas hydrophilic PSs have been entrapped in large unilamellar vesicles (100-500 nm) because of their large aqueous core (9,18).

Dermal delivery

Superficial cutaneous tumor lesions are directly accessible for topic application of PS molecules onto the disease site (12), minimizing eventual concerns involved in intravenous administration. Nevertheless, the efficient penetration of the PS into skin layers is essential to achieve successful PDT results (12). Some studies have shown that liposomal formulations, associated with organic solvents, terpenes, edge activators (sodium cholate, polysorbate 80 or polysorbate 20) and ethanol, enhance PS penetration through the skin, even to an extent comparable to that of subcutaneous administration (12,19). This enhanced penetration feature is attributed to the high elasticity, small size and high flexibility shown by these vesicles, which are therefore able to squeeze out through narrow constrictions of the skin layers and/or to interact with skin stratum corneum lipids, to solubilize these molecules and to create some channels enabling their permeation through the skin (12,19).

Fang et al. (20), using an animal skin permeation protocol, evaluated ethosomes - liposomes containing a relatively high concentration of ethanol - as PS dermal delivery systems. The PS used was ALA, a precursor of the PS protoporphyrin IX (PpIX), formed *in vivo* after exogenous ALA application. The results showed that PpIX concentration in the inner skin layers was enhanced when ALA was carried by ethosomes compared to conventional liposomes. The same group investigated the permeation ability of topically applied ALA-containing ethosomes in a hyperproliferative mouse skin model. Ethosome vehicle formulation showed a 3.64-fold increase in PpIX detection in mouse skin when compared to ALA aqueous formulation vehicle (20). Dragicevic-Curic et al. (21) showed that the PS meta-tetrahydroxyphenylchlorin (mTHPC) associated with conventional liposomes showed lower skin penetration than ethosomes formulated with 20% ethanol. The same research group also reported a significant reduction in the

growth of the very invasive human colorectal carcinoma HT29 in mice after topical application of mTHPC associated with liposomes obtained by a combination of phospholipids, ethanol and terpenes (12).

In order to improve dermal delivery, the association of liposome with ionophoresis (to enhance the flux of ionic compounds across the skin) has been suggested (22). Taken together, all the experiments described here show that it is possible to improve the topical delivery of PSs simply by incorporating new components into the liposome formulation, once more proving the versatility of this drug delivery system. Other liposomal formulations for topical drug delivery and their related patents are described elsewhere (23).

Intravenous delivery

The interaction of conventional liposomes with a diseased tissue after intravenous administration is not immediate. Once administered by the intravenous route, the liposomes are exposed to several proteins, cells and tissues that can reduce or even totally prevent them from reaching their target site. For example, it is known that liposomes are rapidly eliminated from the bloodstream due to surface opsonization and later phagocytosis by the reticuloendothelial system (RES), a physiological system mainly consisting of macrophages resident in the liver, spleen and lymphatic system, responsible for the elimination of several macromolecules and particles from the body (22,24). Moreover, the interaction of liposomes with plasma lipoproteins - which destabilize the vesicles - significantly reduces the circulation time of liposomes in the bloodstream and promotes the leakage of the PS before it can reach the target site (17).

The knowledge about the dynamics of particles administered to the human body has led many researchers to suggest interesting modifications in the structure/composition of liposomes in order to improve not only their stability but also their tissue selectivity and internalization. Particularly, there are some well-known questions regarding both the vasculature and the lymphatic clearance of particles at the tumor site that have created an entire line of improvements in antineoplastic liposome formulations.

It is known that tumors are supplied by leaky vessels presenting gaps between their endothelial cells (25). Because of this leaky vasculature, compounds and particles tend to migrate easily from the bloodstream to the diseased tissue. Furthermore, the presence of poor lymphatic drainage in these lesions also contributes to the enhanced retention time of the particles. Both the increased permeation and reduced clearance of particles in the tumor account for the passive accumulation of particles at the disease site, known as the 'enhanced permeability and retention' (EPR) effect (26). When the PSs are associated with liposomes ranging in size from 100 to 400 nm, the passive PS accumulation

in the disease tissue is optimized (27).

Nevertheless, the short circulation time of conventional liposomes (tens of minutes) in the bloodstream impairs their efficient accumulation at the disease site, which depends on the frequency at which the liposomes pass through the tumoral vessels. Some surface modifications have been suggested in order to reduce the interaction of liposomes with the immune system. Studies have demonstrated that the addition of a polymeric hydrophilic coat to the liposome surface significantly increases its half-life in the bloodstream by reducing the recognition and interaction with plasma proteins and RES macrophages (28,29). These long-circulation liposomes are known as PEGylated liposomes, due to the use of polyethylene glycol as a coating agent. In fact, PEGylated liposomes have an extended half-life in the bloodstream in comparison to conventional liposomes and subsequently enhanced PS accumulation in diseased tissues by the EPR effect, as observed by Sadzuka et al. (30). These investigators reported a higher accumulation rate of the PS coproporphyrin I incorporated into PEGylated liposomes and an improved response to PDT treatment in mice when compared to conventional liposomes and/or to free PS. Similarly, Oku et al. (31) showed that 80% of mice bearing a subcutaneous sarcoma were cured after PDT treatment with intravenous injection of the benzoporphyrin derivative monoacid ring A (BPD-MA) incorporated into glucuronide-modified liposomes, while only a 20% cure rate was observed for injected PS-conventional liposomes.

Selectivity and dose improvement

Besides prolonged bloodstream circulation time and notable tumor accumulation, some reports have claimed that the cell interaction/uptake of PS-PEGylated liposomes is low and consequently may lead to reduced PDT efficiency. For instance, Oku et al. (31) reported that the PEGylation of BPD-MA-liposomes enhanced the passive accumulation of the liposomal drug in tumor tissues at 3 h after administration, but did not enhance the PDT efficacy, suggesting that the liposomes were not effectively taken up by the tumor cells before laser irradiation. Moreover, the low interaction of PEGylated liposomes with cells also reduces the amount of reactive oxygen species (ROS) that reach the targeted cells, since ROS are generated at the PS activation site and then diffuse through short distances (about 0.02 µm) due to their short half-life in biological tissues (3).

Besides the passive accumulation obtained by the EPR effect with liposomes within a certain size range, the conjugation of targeting molecules to the liposomal surface is an interesting alternative for obtaining a more specific localization of the PS, not only improving tissue selectivity but also reducing the effective PS concentration necessary to achieve successful PDT results (32). In contrast to the passive accumulation process, this approach is known as active targeting and is mainly used to specifically detect

tumor cells and vessels. Both the passive and active targeting can work together in accumulating the PS-containing liposome at the tumor site and specifically directing the PS to the tumor cells, respectively.

The active targeting to tumor cells can be based on the fact that tumor cells aberrantly overexpress certain tumor-associated antigens that are found in low amounts or are even absent in non-tumor cells. Tumor cells overexpress, for example, receptors for vitamins (folate), growth factors, glycoproteins (transferrin) and glycolipids to support their high metabolic activity (7). Liposomes conjugated with these tumor-specific ligands have been developed for PS delivery in antineoplastic PDT. The treatment of cervical cancer cells overexpressing transferrin receptors with PEGylated liposomes conjugated with transferrin significantly increased the PS AlPcS4 cell uptake and was ten times more effective than the free AlPcS4 (33).

Several tumor-specific ligands are widely available, relatively inexpensive and minimally immunogenic (17). Nevertheless, their use demands a careful evaluation of the overexpressed receptors present in the tumor microenvironment to avoid reaching normal cells that often express receptors for the same ligands (17).

Internalization improvement

After the initial interaction of the liposome with the surface of the target cell, it is important that PS reaches the subcellular location that causes the desired damage to the target cell, be it cell death by apoptosis or by necrosis, or even just cellular stress. Internalization, instead of just adsorption, of the PS-containing liposomes by the cell is generally desired in order to induce more intense cell damage. Most of the internalization strategies are focused on endocytotic processes, such as phagocytosis, or on the invading mechanisms used by some intracellular parasites.

Viruses, for example, are endowed with fusogenic proteins that are able to bypass the plasma membrane allowing the internalization of their genetic material (34). Fusogenic viral envelope proteins and cell penetrating peptides, such as TAT (from the HIV virus), are positively charged molecules able to adhere to the target cell by electrostatic attraction since the cell membrane possesses an overall negative charge (35). This viral strategy has inspired researchers to design liposomes containing cationic lipids or cationic peptides/polymers coupled to their surface, which have been shown to be interesting models for the delivery of PS due to the enhanced effective tissue penetration (7).

Molinari et al. (36) reported that the internalization of the PS m-THPC in malignant glioma cells was significantly enhanced when cationic liposomes were used as carriers. Takeuchi et al. (37) designed a cationic polyethylenimine-modified liposome encapsulating the PS BPD-MA for anti-angiogenic PDT and reported that the cationic liposomes maintained their phototoxicity to endothelial cells while the

effect of non-modified liposomes was suppressed (37,38). Another study by the same research group also reported the destruction of angiogenic vessels and the subsequent apoptosis of tumor cells after *iv* injection of a low dose of cationic BPD-MA-liposomes on sarcoma-bearing mice (39).

Although the internalization mechanism for liposomes is not fully known, the process of endocytosis has been described as one of the most common pathways by which these particles are internalized by cells (35,40). A remarkable characteristic of this process, which must be taken into account when designing PS-containing liposomes, is that endosome vesicles entrapping liposomes fuse with the lysosome, which contains enzymes in an acid aqueous environment, before the PS is released in significant amounts to the cytosol (40). Thus, the endosomal/lysosomal pathway can potentially damage the PS and, consequently, reduce their effectiveness. In this context, a potential advantage of cationic liposomes, which is also explored by some viruses, is that they can fuse with the endosome membrane and then release their content directly into the cytoplasm, preventing it from being degraded by lysosomal enzymes (35).

The use of cationic liposomes increases the efficiency of S internalization for PDT treatment, thus reducing the total therapeutic dose needed. Nevertheless, cationic liposomes do not present any selectivity for specific cells - unless that mediated by surface charge - and they may in fact fuse with almost all types of mammalian cells (35). In order to obtain tumor selectivity and assure low side effects, the combination of cationic molecules and active-targeting moieties is necessary (41). In addition, the exact proportion of positively charged polymers and/or phospholipids to be added to a liposome formulation must be correctly calculated, since the fraction of positively charged molecules in a liposome is likely to be critical for its effective delivery, cell uptake and toxicity (35).

Liposomal formulations currently in clinical use

Several pharmaceutical companies are investigating new PSs, PS delivery vehicles, and PDT protocols for the treatment of different tumors (3,7). Only one liposomal formulation has been approved thus far by the FDA for clinical application in PDT, but it is not designed for antineoplastic treatment. This formulation, with the registered trademark Visudyne®, contains BPD-MA, verteporfin and a 1:1 mixture of the structural isomers BPD-MA$_C$ and BPD-MA$_D$ (42). It is a reconstitutable lyophilized powder for intravenous infusion, indicated for the treatment of age-related macular degeneration in patients with predominantly classic subfoveal choroidal neovascularization. The rationale of the Visudyne® therapy relies on the elimination of the neovasculature - responsible for the macular degeneration - after vascular occlusion elicited by the endothelial damage

caused by the ROS generated after photoactivation of the liposomal PS (42).

New perspectives

PDT and hyperthermia

Tumor cells are more sensitive to temperatures in the range of 38-45°C than normal cells. This characteristic has led to the development of hyperthermia therapy, which is based on the heating of tumor tissue in order to kill neoplastic cells. The association of PDT and hyperthermia in a single therapy has been proposed and encouraging results have already been published (43). This is an interesting approach because one treatment complements the other as oxygenated tumor cells are preferentially killed by PDT while hypoxic cells are preferentially killed by hyperthermia (44,45). Therefore, besides the advantages of liposomes in PDT itself, this type of drug delivery system could also be used to carry hyperthermia agents such as superparamagnetic nanoparticles, associated with PSs, an approach that has a great potential to improve tumor cell death rates. Nevertheless, to our knowledge, such studies employing liposomes still remain to be done.

Magnetoliposomes

The entrapment of magnetic nanoparticles into PS-containing liposome carriers is another promising approach to tissue-specific PS targeting, given that a magnetic field can be used to selectively accumulate the PS-magnetic liposome in a certain tissue (22). Oliveira et al. (46) described the characterization of a magnetic liposome entrapping the PS zinc phthalocyanine and reported that the photophysical and photochemical properties of the PS were very close to the characteristics of an ideal PS compound. Aside from encouraging results, the effects of PS-magnetic liposomes in *in vivo* tumor models have not been reported, indicating that further improvements are needed for this kind of liposomal formulations.

Immunization

Most of the antineoplastic treatments induce a large destruction of the tumoral mass, but generally they are not capable of totally eliminating it, often due to the tumor idiosyncrasy or to the limited range of treatment. PDT itself can lethally damage over 90% of the neoplastic cells in tumors (47), but the remaining viable neoplastic cells are still capable of maintaining the tumor growth after PDT, representing a potential possibility for tumor recurrence. Taking into account only this acute effect, PDT could be viewed only as a co-therapy but not as a main antineoplastic approach. However, the effects of PDT go far beyond the more discussed light-induced oxidative damage in the cells; they do affect the structure and function of the tissue in which the cell is located and, often, the whole organism.

The cellular debris along with the inflammatory environ-ment elicited at the site of PDT application are responsible for a potential long-lasting effect, which can be more important than the direct cell killing itself, i.e., the activation of the adaptive immune system against tumoral antigens (47). This event can be responsible for the destruction not only of all neoplastic cells in the treated tumor, but also of those metastatic neoplastic cells sharing antigens with the PDT-damaged ones. Experimental evidence supports this rationale (48). The classical study by Korbelik et al. (49) showed that, although the acute antineoplastic PDT effect was the same in both immunocompetent and immunodeficient individuals, the long-term protection against tumor recurrence was obtained only in immunocompetent mice, demonstrating the crucial role played by the immune system after PDT application.

The mechanisms behind the PDT-induced immunization are not fully understood but several studies relate cellular stress and damage to the subsequent immunization. The cellular debris at the site of PDT application are strong inducers of the immune system and their immune adjuvanticity seems to be dependent on the danger signals, which are discussed in detail elsewhere (50,51). The danger signals are often related to cell stress/damage and are capable of eliciting strong inflammatory responses (52). The cells subjected to PDT undergo a characteristic stress response, increasing the expression of stress molecules, such as heat-shock proteins (53). The lysis of these stressed cells occurs within minutes to hours after PDT application, releasing several stress proteins along with other intracellular components such as ATP, which function as danger signals. These danger signals promote the activation of the dendritic cells (DCs) present at this site, which then become capable of migrating to the draining lymph nodes and activating effector functions on T cells specific for the tumoral antigens (47,50). In addition to DCs, other antigen-presenting cells at the PDT application site can be responsible for the local reinforced activation of the adaptive immune system.

Another significant event following PDT is the direct ROS-mediated activation of inflammatory signaling pathways, such as those leading to the formation of inflammasome complexes (47,52), which play a critical role in immunization. The production of ROS can be directly or indirectly promoted by PDT. The direct stimulation is related to the classical generation of singlet oxygen after PS photoactivation, previously cited as Type II reaction. By the indirect pathway, the cellular debris, along with the danger signals released by damaged cells, elicit the oxidative burst by inflammatory cells (47). Therefore, both the PDT-induced oxidative burst and the danger signals can be viewed as important adjuvants for the process of immunization.

However, despite the important immune adjuvanticity exerted by the cellular debris, some studies have related the local application of exogenous immune adjuvants to a significant improvement of PDT-induced tumor immunization (54). This finding suggests that formulations containing

not only photosensitizer but also strong immune adjuvants could improve the long-term effectiveness of PDT and also relating the use of immunoadjuvants with an improvement in the antineoplastic PDT (47,54).

Liposomes have proven to be good adjuvants and antigen vehicles for vaccines. Therefore, the use of liposomes in formulations designed for antineoplastic PDT applications can make it possible to concentrate both the PS and immunoadjuvants at the tumor site, eliciting the immunization against tumoral antigens. This is an alternative for the development of a rational antineoplastic PDT-based immunization protocol in order to increase the long-term effectiveness of antineoplastic PDT.

As discussed throughout this review, the use of liposome nano-carriers may be a suitable strategy to improve PS effectiveness in PDT application. Due to its lipophilic nature, PS in association with liposomes can be useful to prevent PS aggregation in aqueous solutions and to increase PS delivery to tumor tissues by both passive (according to the diameter of vascular pores) and active (association with target molecules) accumulation. Furthermore, new perspectives point out the association of PS with different antineoplastic agents, such as hyperthermia agents or immunoadjuvants, in the same liposomal formulation. Taken together, all the facts discussed here indicate that PS-containing liposomes are a viable pharmaceutical option to improve both the safety and efficacy of antineoplastic PDT.

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