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Therapeutic Delivery

Development of nanosystems for active tumor targeting in photodynamic therapy

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"A very productive period for nanomedicine will arrive in the next few years that will probably generate a large number of innovations to translate from bench-to-bedside."

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According to the International Agency for Research on Cancer, an estimated 19.3 million new cancer cases and almost 10 million cancer deaths occurred in 2020 [1]. Furthermore, the diagnosis and treatment of cancer have been hampered by the coronavirus disease 2019 (COVID-19) pandemic, which is expected to result in an increased cancer mortality over the next years due to delays in diagnoses and due to the interruption of cancer treatments. For example, the US National Cancer Institute estimated a 1% increase in deaths related to breast and colorectal cancer over the next 10 years, the equivalent of approximately 10,000 more deaths, due to the impact of the pandemic [2]. This highlights the need to continue in the search for new therapeutic compounds in order to reduce the chance of cancer recurrence after traditional treatments such as surgery and radiotherapy.

Some of these new treatments could also be used as primary or adjuvant therapeutic options. For instance, photodynamic therapy (PDT) arises as an improved treatment tool due to its highly effective, noninvasive and localized therapeutic action. Taking into consideration the localized irradiation of the tumor area with PDT, that it does not compromise other treatment options, and that it presents reduced long-term morbidity when compared with chemotherapy or radiotherapy, this photo-assisted therapy is positioned as a main and/or adjuvant treatment in the fight against cancer. PDT has been approved by the US FDA, and also by other regulatory agencies around the world, to treat a variety of tumors and malignancies in the clinic [3]. For the success of PDT, three elements must converge in tumor cells: photosensitizer (PS) accumulation, light irradiation penetration and the presence of molecular oxygen. More of the recent developments regarding PDT have been made around the generation of new PSs.

A PS is a molecule capable of absorbing photons to produce excited species that react with molecular oxygen (in a ground state form of triplet oxygen, $^3O_2$) or biological cell components, either through energy transfer or electron transfer processes, to produce reactive oxygen species (ROS) with special emphasis on the generation of singlet oxygen ($^1O_2$) as the main unstable and reactive ROS to oxidize biomolecules. ROS can damage various cellular components including lipids, DNA and proteins and can also generate an imbalance in the basal redox state of cells, which leads to the cell death.

PSs are classified into three generations based on their evolution. First-generation PSs such as porphyrin and hematoporphyrin are the most accepted and approved ones for clinical use but are considered to have low selectivity, high skin photosensitivity and low molar extinction coefficient due to their physicochemical characteristics [4]. The second-generation of PSs was developed with the aim to improve the characteristics of the first generation, such as the ROS yield of formation and the wavelength of light that activates them (which translates to deeper light tissue penetration). These new PSs are obtained as one single pure molecule either by total synthesis or by hemisynthesis from an existing PS. These PSs include chlorins, phthalocyanines and porphyrin derivatives, which were superior

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to the first-generation PSs in favoring the formation of the PS excited triplet state, leading to an increase in the quantum yield of $^1O_2$ formation. However, second-generation PSs are mostly hydrophobic and some of them are not suitable to be conjugated with monoclonal antibodies or other small targeting molecules to enhance the specific uptake by tumor cells [4]. Therefore, problems such as lack of selectivity, poor water-solubility, possible toxic effects on healthy tissues and skin photosensitivity may prevent the clinical applications of various PDT PSs and for these reasons, a third-generation of PSs has arrived in recent decades.

The development of new drug delivery systems with high therapeutic efficacy linked with low side effects-compared with traditional chemotherapy drugs-is a challenge in the biomedical field. Nanomedicine is a promising area to explore for new therapeutic drugs and after the development of Doxil®, a liposome nanocarrier containing doxorubicin, several articles were published that focus on the design of novel nanosystems to treat oncological conditions and their evaluation by *in vitro* biological assays and in preclinical trials, some of them by our group [5–10]. At present, research focus on the development of nanoparticulate systems has led to numerous innovations in the treatment of cancer and is revolutionizing the delivery and enhancing the effectiveness of biologically active molecules, at least in preclinical trials.

Based on PDT research literature, several efforts are being made to improve features of second-generation of PSs or to create new ones with multiple features including active targeting for a better selectivity of PSs. A number of approaches has been explored to produce nanoparticles (NPs) with the ability to transport different cytotoxic agents as well as PSs affording PDT-based synergistic therapies for improved response rates-to decrease the disadvantages and overcome obstacles presented by PDT-that will probably lead to a resistance by tumor cells after treatment [11]. In this sense, various kinds of NPs have been developed using different materials such as polymer, lipid, gold, silica, carbon and iron oxide, which have shown promising results in many research papers to efficiently deliver PSs to tumor tissue, add other molecular damage mechanisms or even increase the PDT performance. As a first attempt, PSs have been physically loaded or chemically conjugated to NPs and because many PSs are hydrophobic, they are contained in the hydrophobic regions of self-assembled NPs. For this purpose, a few biocompatible polymers had been used to encapsulate PSs such as poly(D,L-Lactide-co-glycolide) (PLGA), poly(glycolide) (PGA), poly(D,L-Lactide) (PLA) and polyethylene glycol. The pharmacodynamics and bioavailabilities of these different polymer nanoparticles may differ from one another, thus the efficiency of PDT may be different according to the nature of the polymer [12,13].

Son and coworkers have reported a comparative study encapsulating two well-known PSs-chlorin e6 (C6) and pheophorbide (Pba)-in PLGA NPs [14]. Ce6 is more hydrophilic than Pba, and after intravenous (iv.) administration in tumor bearing mice, Pba was more efficiently delivered to tumor tissue by the same NPs compared with Ce6 due to the stable loading of the more hydrophobic Pba. In a similar approach, our group developed conjugated polymer nanoparticles (CPNs), which were improved in their ability to generate $^1O_2$ by the incorporation of an organic hydrophobic porphyrin-platinum octaethylporphyrin-for the efficient photokilling of glioblastoma tumor cells [15]. As it was mentioned before, $^1O_2$ quantum yield of formation is used as an explicit PDT dosimetry quantity of efficacy because $^1O_2$ is the major cytotoxic ROS agent; the half-life of $^1O_2$ is shorter than other ROS and; therefore, the damage generated is more localized to the tumor cell components, which improves the treatment efficacy. With a theranostic approach, the incorporation of magnetic iron oxide nanoparticles into the CPNs allowed the distribution of nanoparticles after iv. administration, and therefore the PDT itself, to be followed by MRI. Additionally, the cytotoxicity of CPNs was not affected by the iron oxide nanoparticles incorporation [9]. However, the tumor accumulation of these types of PSs needs to be improved by the modification of the surface of the NPs with biological ligands with affinity for specific receptors on target cells to obtain more selective tumor attack. On the other hand, passive targeting is achieved by using any kind of nanoparticles, due to the well-described phenomenon called the enhanced permeability retention effect which allows the passive accumulation of NPs into the tumor [16]. However, the amount of nanoparticles that reach the tumor tissue is only around approximately 1% of the injected NP dose (% ID) [17].

Antibodies, peptides, aptamers and small molecules have been used for active targeting of nanoparticulate PSs to tumor cells and other cells from the tumor microenvironment (TME) as well as neovessels surrounding the tumor [18,19]. Putting aside the classical selective attack on tumor cells with PDT, a few efforts have been made to photokill other cellular components of the TME that sustain tumor growth. For example, bovine serum albumin was employed to synthesize curcumin-loaded bovine serum albumin nanoparticles, which are highly biocompatible and nontoxic and were used to eliminate cancer stem-like cells (CSCs) [20]. In another study, the selectivity of PSs for CSCs was achieved by engineering cellular radiative transfer energy between leucine-rich repeat-containing

G-protein coupled receptor 5 GFP-expressing cells and rose Bengal PS [21]. In these reports, the selective destruction of CSCs was not only achieved with the addition of ligands but also due to the selective spatial irradiation of PDT.

In another approach, Katsube *et al.* demonstrated that using a fibroblast activation protein-targeted near infrared photoimmunotherapy, which utilizes near-infrared irradiation and the ability of fibroblast activation protein-α to recognize cancer-associated fibroblasts, it is feasible to overcome the resistance to conventional chemotherapy in esophageal cancer by eliminating cancer-associated fibroblasts [22]. Many other reports have been published evaluating more selective PDT with the purpose of overcoming some resistance factors from TME, such as other cellular or acellular components [11]. For instance, Hayashi *et al.* synthesized a mannose-conjugated chlorin (M-chlorin) designed to bind mannose receptors, which are highly expressed on tumor associated macrophages [23]. With this approach, M-chlorin PDT revealed strong cytotoxicity for M2 tumor associated macrophages in the TME *in vitro* and *in vivo*.

In addition to the approach of active targeting mediated by the surface modification of NPs with biomolecules, other more biomimetic approaches correlated with biological events, such as the intercellular communication by vesicles and cell migration and recruitment into tumors, have been reported in the literature to deliver NPs. Trojan horse cellular delivery using circulatory cells as drug delivery vehicles was successfully evaluated to carry NPs into tumors and increase PDT efficacy [10,24]. In a more sophisticated way, cell-membrane-coated nanoparticles have emerged as a promising antitumor therapeutic strategy and could be combined with PDT to generate direct damage to tumor cells or induce immunogenic cell death and enhance the antitumor immunity efficiency of T cells in distant tumor cells such as those from a metastasis [25].

Finally, it is necessary to mention that effective cytotoxic effects of PDT can be obtained by localization of PSs in intracellular organelles such as mitochondria, the nucleus and lysosomes because of the reactivity and short half-life of the ROS generated by light irradiation. In order to allow PSs to accumulate in the target organelles in selective cells at adequate concentrations, modification of PSs has been achieved using cell-penetrating peptides or conjugation with targeting ligands [26].

In short, based on the diverse and increasing literature regarding PDT tumor active targeting, different strategies have been studied with the purpose of increasing PS solubility and achieving binding to target cells and organelles based on the cell type or cell component that is to be damaged or eradicated. The selectivity of PDT could be achieved not only in tumor cells, and TME cell components are of particular interest to target to increase therapeutic efficacy. A very productive period for nanomedicine will arrive in the next few years that will probably generate a large number of innovations to translate from bench-to-bedside. It is important to highlight that dialogue among the different sectors of the innovation ecosystem, including academia and industry, is essential to overcome regulatory and commercial obstacles to finally obtain clinical products for the benefit of the entire population.

Author contributions

LE Ibarra was responsible for the conception or design of the work; acquisition, analysis and interpretation of data; drafting the work and revising it critically and final approval of the version to be published.

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LE Ibarra is a member of the Scientific Researcher Career at CONICET and faculty at UNRC, Argentina.

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