Stimuli-Responsive Systems of Therapeutics

Review

Nanoparticle-Based Photodynamic Therapy:
Current Status and Future Application to
Improve Outcomes of Cancer Treatment

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Photodynamic therapy (PDT) is an emerging cancer treatment that uses photosensitizers (PS), along with light to activate them, resulting in oxidation of various biological components in cancer tissues. However, since most potential PS are solubilized and given as aqueous solution, PS is non-specifically distributed in the body, leading to the induction of various side effects in normal tissues that are exposed to daylight such as skin and eyes. To overcome the problem associated with non-specific in vivo disposition of PS, various approaches have been applied to develop safer dosage forms for PS with more efficient tumor delivery and lower disposition to normal tissues. Passive drug targeting to tumors with nanoparticulate formulations has been recognized as one of the potentially useful approaches to improve the poor tissue specificity of conventional cancer chemotherapy and this approach should also be applicable for more efficient tumor delivery of PS. In this review article, several issues concerning the efficacy of PDT using nanoparticle-based formulations are discussed and our recent attempts to temporally enhance the vascular permeability within tumors with photodynamic treatment for the better therapeutic outcome of nanoparticle-based therapy are introduced.

Key words  photodynamic therapy (PDT); nanoparticle; drug delivery; photosensitizer (PS); vascular permeability

1. Introduction

The leading cause of death worldwide is cancer. Cancer deaths are expected to continue to rise, with an estimated 11.4 million mortalities in 2030. To develop more effective therapeutic modalities for cancer, improvement of our knowledge of cancer (patho)physiology, discovery of new drugs, and development of novel bio-medical technologies are necessary. Among these challenges, effective delivery of drugs in the active form to solid tumors is highly important. Most small-molecular chemotherapeutic drugs non-specifically distribute throughout the body after intravenous administration, and this often results in a narrow therapeutic index due to their toxicities in normal tissues. Thus, the in vivo disposition of anti-cancer drugs should be improved and the toxicity of current chemotherapy decreased to extend survival time and improve quality of life of cancer patients.

Photodynamic therapy (PDT) is a new cancer treatment modality that uses photosensitizers (PS), along with light to activate them, resulting in oxidation of various biological components in cancer tissues.1,2) Basically, PDT as a cancer treatment is performed as follows: (i) topical or systemic administration of PS; and (ii) irradiation of non-thermal light (635–760 nm) to tumor tissues, leading to generation of reactive oxygen species (ROS) resulting in the damage of cancer cells. This treatment can provide a certain tissue selectivity; however, like other cancer chemotherapies, PDT has several limitations. First, since most potential PS are poorly water soluble and easily aggregated after administration, such a physicochemical property of PS makes it very difficult to formulate them adequately and sometimes extremely decreases their photodynamic activity against tumors.3) Second, even if PS is adequately solubilized and given as solution, PS is non-specifically distributed throughout the body, leading to induction of various side effects in normal tissues that are exposed to daylight such as skin and eyes. Skin photosensitivity reactions such as hyperpigmentation, erythema, edema, and blistering significantly reduce quality of life of patients receiving PDT.4)

Passive drug targeting to tumors with nanoparticulate formulations is recognized as one of the potential approaches to improve poor tissue specificity of conventional cancer chemotherapy.5,6) This approach exploits the leaky tumor vasculature and impaired lymphatic drainage, allowing nanoparticulate formulations to selectively access and accumulate into tumors, so-called enhanced permeability and retention (EPR) effect7–9) (Fig. 1). Passive drug targeting to tumors using nanoparticulate carriers takes advantage of these unique physiological characteristics of tumor vasculature. It has been demonstrated that anti-cancer drugs formulated in polyethylene glycol (PEG)-modified liposomes preferentially accumulate in tumors,10) and doxorubicin encapsulated in PEG liposomes or Doxil has been approved and is widely used for patients with Kaposi sarcoma and ovarian cancers.11) The EPR effect-driven

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tumor targeting of nanoparticles, may also be suitable for selective delivery of PS encapsulated in nanoparticles. In this review article, several issues concerning the efficacy of PDT using nanoparticle-based formulations are discussed and our recent attempts to temporally enhance tumor vascular permeability with photodynamic treatment and thereby improve outcomes of nanoparticle-based therapy are introduced.

2. Mode of Action of PDT in Cancer Treatment

As already described above, PDT as a cancer treatment is performed by two sequential treatments: PS administration; and irradiation of non-thermal light to tumors. Subsequently, the relatively long-lived excited triplet state of PS is generated via inter-system crossing from a fraction of the excited singlet state. Finally, singlet oxygen (\( ^1O_2 \)), a primary phototoxic species generated upon the light irradiation, including other reactive oxygen intermediates such as superoxide radical (\( O_2^{-} \)), hydroxyl radical (\( OH^- \)) and hydrogen peroxide (\( H_2O_2 \)) are formed by the interaction of the excited triplet PS with molecular oxygen. These ROS first induce impairment of cellular function and structure, and these damages eventually result in death of cancer cells and hence tumor growth inhibition.\(^{2,3}\) Since ROS are generated only when PS, light with certain wavelength, and oxygen molecules coexist, tumor selectivity can be expected to a certain extent.\(^{13,14}\) Furthermore, in contrast to other conventional cancer chemotherapies, acquisition of drug resistance is less likely expected and therefore repeated treatments may be possible.\(^{15}\)

3. Nano-DDS Formulations for PS and Their Delivery into Tumor Tissues

PDT-based cancer treatment has several promising advantages as described in the previous section, but there are also potential problems related to the solubility and in vivo disposition characteristics of PS given as solution. Indeed, although several potent PS are now being developed, they are difficult to administer, especially in the case of intravenous injection, because of their high lipophilicity. To address these problems, various approaches have been applied to develop safer dosage forms for PS with higher solubilizing capability, more efficient tumor delivery and lower disposition to normal tissues. Hence, the development of better delivery systems for PS is critical to improve outcomes and realize application of PDT in clinical settings.

Several approaches are currently under development, among which nanoparticle-based formulations can offer several advantages as follows. For instance, nanoparticle-based formulations can provide high loading efficiency and controlled release of PS. A suitable material or manufacturing process can be rationally chosen for a given PS. Furthermore, as described above, nanoparticle-based formulations can make passive drug targeting approaches possible by exploiting unique physiological characteristics of tumor tissues.\(^{8,9}\) Although different types of nanoparticulate formulations for PS including liposome,\(^{16,17}\) emulsion,\(^{18}\) and others\(^{19,20}\) have been studied and all of these formulations improved PS solubility to some extent (Table 1), their achievement, especially their in vivo performance is still limited. Among nanoparticulate carriers available, polymeric nanoparticles (PN) composed of an amphipathic diblock copolymer would be one of suitable drug carriers for PS.\(^{21,22}\) PN possess hydrophilic outer shell associated with an aqueous layer that protects the interaction of PN with plasma proteins such as opsonins enhancing phagocytosis by macrophages in the liver and spleen. Avoidance of removal by the reticuloendothelial system would lead to prolonged circulation in blood, a property that is essential for efficient EPR effect-driven tumor delivery of PN. Furthermore, PN has a hydrophobic core wherein lipophilic PS can be readily encapsulated.

Based on these structural and functional advantages that PN can provide, a hydrophobic porphyrin derivative, photo-protoporphyrin IX dimethyl ester (PpIX-DME), was formulated into polymeric nanoparticles composed of polyethylene glycol and polyactic acid block copolymer (PN-Por) in our previous study.\(^{23}\) As expected, PN-Por exhibited favorable in vivo disposition characteristics after intravenous injection such as prolonged circulation in blood due to its low hepatic and splenic disposition, and this long residence in blood circulation resulted in efficient tumor disposition of PN-Por in Colon-26 (C26) tumor-bearing mice. Furthermore, local light
irradiation onto C26 tumor tissues after PN-Por injection led to potent tumor growth inhibition in these animals. These results suggest that strategies that use nanoparticulate formulations such as PN-Por may be promising for developing more effective PDT-based cancer treatments.23)

4. Determinants of EPR Effect-Driven Tumor Delivery of Nanoparticles

Considering that the above-mentioned tumor delivery of nanoparticles is dependent on extravasation of nanoparticles through tumor vessels with enhanced permeability, differences in these pathophysiological characteristics of vasculature among various types of tumors may lead to different therapeutic outcomes. Our group previously tried to clarify the determinants for in vivo anti-tumor efficacy of PEG liposomal doxorubicin (DOX) by examining its anti-tumor effects against three types of tumor cells with different phenotypes [C26, Lewis lung cancer (LLC), and B16BL6 melanoma (B16)].24) Although C26 showed the lowest sensitivity to DOX in vitro, the most prominent anti-tumor effect of PEG liposomal DOX was observed in C26 tumor-bearing mice in an in vivo study. Based on the highest disposition of PEG liposomes in C26 tumors, efficient tumor delivery should be one of the major factors determining the in vivo anti-tumor effects of PEG liposomal DOX. Our results also indicate that although the extent of vascularity was not directly related to the tumor disposition of PEG liposomes, the vascular permeability within tumors substantially affected the tumor disposition of PEG liposomes and would be one of the major factors determining in vivo anti-tumor efficacy of PEG liposomal DOX, which was supported by the finding that the vascular permeability within C26 tumors assessed by Evans-blue extravasation assay was higher than that within other types of tumors.24)

Efficacy in the delivery of nanoparticles to pancreatic tumors is known to be very low25) due to their poor vascular permeability compared with other solid tumors.26) Thus, establishment of methods to enhance the vascular permeability within tumors may be highly beneficial to improve efficacy in the delivery of nanoparticles and the subsequent outcome of nanoparticle-based therapy for refractory cancer types such as pancreatic tumors.

5. Cellular Target of PDT: Direct and Indirect Actions toward Tumor Cells

Three different mechanisms have been recognized behind PDT-based tumor growth inhibition. First, ROS generated by PDT can directly kill tumor cells by causing apoptotic and/or necrotic cell death. Second, PDT damages endothelial cells, which can lead to thrombosis and/or hemorrhage in tumor vasculature, resulting in death of surrounding tumor cells via the lack of nutrients and/or oxygen. Thus, collapse of existing vessels and/or inhibiting vessel formation (angiogenesis) can improve efficacy of treatment.27) Third, PDT-driven immune reactions induce secretion of various stress response proteins and subsequent leukocytes invasion, which contributes to tumor destruction as well as the stimulation of immune responses to detect and attack tumor cells even at isolated locations. Among these three different cellular mechanisms, the actions of PDT toward tumor vasculature have attracted considerable attention and are ranging from temporal vascular twitch to long-lasting vessel occlusion, depending on the amount of ROS produced.28) Additionally, PDT has been applied to treat age-related macular degeneration using verteporfin as PS. An earlier study demonstrated that vascular leakage was enhanced shortly after this treatment and lasted for several days, followed by subsequent occlusion of the vessels.29) Considering these backgrounds, it is speculated that if the amount of ROS produced and the site of ROS production is carefully and minutely controlled, a mild but specific effect on tumor vasculature such as a transient enhancement of vascular permeability would be rationally and intentionally extracted instead of permanent vessel occlusion.

6. Application of Photodynamic Treatment to Enhance Tumor Vascular Permeability

To improve the tumor-targeting efficacy of nanoparticles by enhancing the vascular permeability within tumors, various attempts have been made including hyperthermia30) and the use of nitric oxide (NO) donors.31) However, the outcome of these endeavors has been unsatisfactory. Considering the above-mentioned action modes of PDT, application of this modality may be another possible strategy for this purpose. Several factors such as PS dose, the light energy given, and the interval time between PS injection and light irradiation, are known to affect the outcome of PDT.17,32,33) Since PDT with a high PS dose was revealed to cause severe damage to tumor vessels,34) the treatment conditions have to be minutely determined to extract the desired action on the tumor vasculature. In addition, since the lifetime of singlet oxygen produced by light irradiation to PS is very short (dozens of nanoseconds), the area that the molecule can reach within its lifetime is small.35) Hence, light should be irradiated to tumor tissues immediately after PS injection when most of the dose remains in the systemic circulation to ensure the selective and substantial action of singlet oxygen to tumor vasculature.

In our previous study, PDT was intentionally applied to temporally enhance the permeability of vasculature within tumors, the so-called photo-triggered tumor vascular treatment (PVT), and thereby to improve tumor delivery and anti-tumor effect of subsequently injected PEG liposomal paclitaxel (PL-PTX).36) Tumor-selective light irradiation with laser light was conducted for 3 min at 15 min after PN-Por injection (0.1 mg/kg as PpIX-DME), and PL-PTX was intravenously administered into B16 tumor-bearing mice just after light irradiation. As a result, more potent anti-tumor effect was observed, which would be attributed to the higher EPR effect-based tumor disposition of PL-PTX due to the vascular permeability enhanced by reduced pericyte-coverage by PVT36) (Fig. 2).

The mechanism behind the effect of PVT should be clarified to further optimize treatment. Although it was suggested that inflammatory responses such as increased interaction of endothelial cells with leukocytes were involved in enhancement of vascular permeability with the photodynamic treatment,37) a recent report demonstrated that the reduced leukocyte-endothelial cell interaction did not decrease the enhancement of vascular permeability.38) Therefore, the effect of such an inflammatory response is still contradictory and might not be a key factor for success. Another proposed mechanism is that ROS generated in tumor tissues would form or enlarge the inter-cellular gaps between endothelial cells, leading to enhanced permeability of tumor vasculature.39) However, no cor-
relation was obtained between the size of inter-cellular gaps and the vascular permeability of nanoparticles with different sizes ranging from 100 to 300 nm. Thus, further studies are necessary to elucidate the mechanism(s) behind the enhancement of vascular permeability by PVT and to optimize the treatment for more efficient tumor delivery of subsequently injected nanoparticles.

7. Conclusion

To date, nanoparticulate formulations for cancer treatment approved and launched into market are still limited despite the remarkable development and accumulated knowledge in nanotechnology. This is partly due to differences in the (patho)physiological characteristics of the microenvironment of tumor tissues including the phenotype of tumor vasculature depending on the type of tumors. Better understanding of these issues should be of great importance to further improve the outcome of EPR effect-based PDT and PVT-driven transient enhancement of vascular permeability within tumors for enhanced delivery and more efficient anti-tumor effect of subsequently injected nano-DDS formulations encapsulating anti-cancer drugs.

Conflict of Interest  The authors declare no conflict of interest.

References


Fig. 2. Proposed Mechanisms behind Enhanced in Vivo Anti-tumor Effect of PL-PTX Pretreated with PVT in B16 Tumor-Bearing Mice