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Review

Pharmaceutical development, composition and quantitative analysis of phthalocyanine as the photosensitizer for cancer photodynamic therapy

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ABSTRACT

Phthalocyanine (Pc) and its related derivatives are a class of functional materials that are easily activated by the light at a special wavelength. As such photosensitizer, Pc has been applied to photodynamic therapy (PDT), in addition to its broad applications in many fields, for both malignant and benign diseases. One of our long-term research focuses is to develop Pc for cancer therapy. Herein we briefly review mechanisms of action of Pc used for photodynamic therapy, its pharmaceutical development and molecular modification to enhance its drugability and improve its intracellular localization. We also describe the current status of the Pc derivatives under clinical investigation, and analyze the methods used for quantitative analysis of those Pc derivatives.

1. Introduction

Phthalocyanine (Pc, Fig. 1) and its derivatives are widely used as functional materials in many high technique fields, such as data storage, photoelectronic generation, catalyst and so on. Using as photosensitizer in photodynamic therapy (PDT) is the most attractive application of Pc to various clinical trials and has attracted intensive interests since 1990s. Nowadays, there are several phthalocyanines under clinical evaluation. However, there are only a few literature focused on pharmaceutical development and quantitative analysis of clinical phthalocyanines. Herein, we will comprehensively review the current status of Pc-based photosensitizer and our experience in analysis of sulfonic phthalocyanines.

2. Mechanisms of photodynamic therapy and its pharmaceutical development

In the war on cancer, continues progresses have been achieved with the great efforts to treat cancer mortality with drugs with better cure and less adverse effects. PDT is such a minimal invasive treatment which is currently used for malignant or benign diseases [1–4]. The therapy drug used for PDT is known as photosensitizer.

PDT involves two procedures. The first procedure is the topical or systemical administration of a light-sensitive photosensitizer which should be selectively accumulated in the target tissue and/or cells. When the concentration of photosensitizer on the lesion is high enough and has a suitable ratio to adjacent normal tissue cells, the second procedure will be carried out, to deliver a specific wavelength of light to the lesion and activate the accumulated photosensitizer. The excited photosensitizer transfers the energy to surrounding oxygen to generate cytotoxic reactive oxygen species (ROS). The ROS, primarily singlet molecular oxygen ($^1O_2$), is responsible for the cascade of a series of cellular and molecular events that result in selective lesion destruction [5,6]. The biological mechanisms of destroying the targeted lesion involve in direct cell killing of tumor cells (cellular PDT), indirect cell killing by vascular occlusion (vascular PDT), and the response of the immune system [4,5]. Selective accumulation of photosensitizer and controlled administration of light to lesion provide advantages of PDT over the three major treatments of malignant tumor (surgery, radiation therapy and chemotherapy), such as protection of functional structures, excellent cosmetic outcome, the safety of repeated uses, less side effects, fast convergence, and lower economic costs.

The development of photosensitizers plays a very important role in PDT. Therefore, photosensitizer has become an important field of research for both chemical and pharmaceutical sciences. The modern era of PDT started with the discovery of hematoporphyrin (Fig. 1) derivatives, the first generation of photosensitizers [7]. Photofrin® (a purified form of hematoporphyrin derivatives),

Abbreviations: Pc, phthalocyanine; ZnPc, phthalocyanine zinc; AlPcS, sulfonated phthalocyanine aluminum; $ZnPcS_2P_2$, di-(potassium sulfonate)-di-phthalimidomethyl phthalocyanine zinc; PDT, photodynamic therapy; ROS, reactive oxygen species; $^1O_2$, singlet molecular oxygen; scFv, single-chain Fv fragments; RGD, arginine–glycine–aspartic acid; EGFR, epidermal growth factor receptor; OPOC, palmitoyl-oleoyl-phosphatidylcholine; OOPS, di-oleoyl phosphatidylserine; USP, United States Pharmacopoeia; ER, endoplasmic reticulum.

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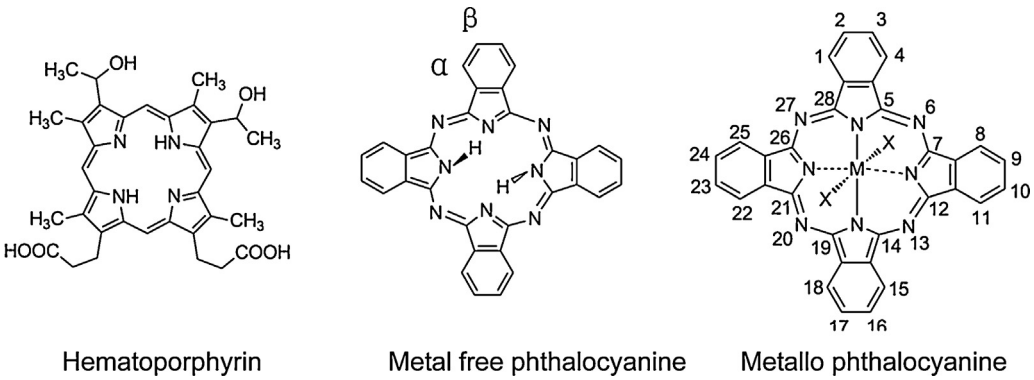

Fig. 1. Chemical structures of representative hematoporphyrin and phthalocyanines.

the first commercial photosensitizer was approved for clinical use for bladder cancer in 1993 in Canada. From then on, PDT has been approved by regulatory authorities of other countries, such as the United States, Japan, the Netherlands and others in the world. PDT has been applied for more indications, such as esophagus, skin, head and neck, and lung cancers. At the same time, PDT is also applied to some non-malignant diseases, such as port wine stains, actinic keratoses, age-related macular degeneration, and localized infection [3,4,8–12].

Although Photofrin® has made considerable success and is still widely used on clinical, it has some shortages, such as moderate efficient and long-lasting skin photosensitization for several weeks, and composition with undefined mixture [7,13]. This led to the development of new generation of photosensitizers. For the design of an ideal photosensitizer, the properties mentioned in the following should be fulfilled, such as: single and chemically pure compound; stability and good solubility in pharmaceutically acceptable formulations and in biological media; high efficiency to yield ROS under illumination; fluorescence; no dark toxicity; fast clearance from the healthy parts of the body and specific retention in diseased tissues; strong absorbance in Near-IR region and minimal absorbance between 400 and 600 nm [10,13,14]. Some photosensitizers of the second generation have been approved at clinical.

3. Pharmaceutical modification of Pc and its derivatives to increase their drugability

Various approaches have been made to improve the pharmaceutical properties of photosensitizers. One logical approach is through conjugation of photosensitizers to various linkers including antibodies, proteins and peptides. Phthalocyanine zinc was first tried to be conjugated to monoclonal antibodies that are specific for tumor-associated antigens [15]. However, the large size of antibodies hinders tissue penetration and lowers cellular uptake when used in vivo. The attachment of photosensitizers to monoclonal antibodies may reduce the antigenic specificity of monoclonal antibodies. As a result, smaller size of biomolecules has been searched for as the more effective alternative. Conjugation of photosensitizers with albumins and low-density lipoproteins has been explored, but only moderate target specificity has been achieved so far [16]. Smaller antibody fragments, such as single-chain Fv fragments (scFv) have received considerable attention as a good candidate [17]. Among various carriers for active drug targeting, synthetic peptides are of particular interest. Peptides with appropriate sequences can specifically bind to different surface biomarkers of cancer cells and circulating tumor cells [18,19]. For instance, two alternative synthetic methods based on Sonogashira cross-coupling of an iodinated phthalocyanine zinc with acetylenic bombesin or arginine–glycine–aspartic acid (RGD) derivatives, either in solution or on solid phase, have been explored to make a series of phthalocyanine zinc conjugates to target the gastrin-releasing peptide and integrin receptors [20]. The peptide conjugation enhanced water solubility of phthalocyanine zinc and improved the latter's photodynamic efficacy against cancer cell lines expressing gastrin-releasing peptide and integrin receptors. Ongarora et al. [21] synthesized four Pc-peptide conjugates for targeting the epidermal growth factor receptor (EGFR) and evaluated the in vitro efficacy of the conjugates by using different cell lines. Two peptide ligands linked to 6 (EGFR-L1) and 13 (EGFR-L2) amino acid residues, respectively. The peptides and Pc-conjugates were shown to bind to EGFR using both theoretical and experimental models. The Pc-EGFR-L1 conjugates efficiently targeted EGFR and were internalized, in part due to their cationic charge, whereas the uncharged Pc-EGFR-L2 conjugates targeted EGFR poorly probably because of their low aqueous solubility. All conjugates were nontoxic (IC_{50} > 100 μM) to HT-29 colon cancer cells, both in the dark and upon light activation (1 J/cm^2). Intravenous administration of the conjugate into nude mice bearing A431 and HT-29 human tumor xenografts resulted in a Near-IR fluorescence signal at 700 nm, 24 h after administration.

To increase the cell-targeting ability, two ZnPc-peptide conjugates, which bore either a short linker or a long PEG-linker between the macrocycle and a bifunctional peptide containing the nucleoplasmin and HIV-1 Tat 48-60 sequences, have been synthesized to evaluate the effect of the linker [22]. The presence of the peptide chain increased the water solubility of the Pc macrocycle and, consequently, its fluorescence in aqueous solutions. The highest fluorescence quantum yields were observed at low pH (5.0) for both conjugates and were always higher for the conjugate bearing the short linker. Both conjugates were found to have low dark cytotoxicity toward human HepG2 cells (IC_{50} 77 μM) but were highly phototoxic (IC_{50} < 2 μM at 1 J/cm^2).

An alternative strategy to improve pharmaceutical properties of Pc involves direct formulations of Pc to increase its solubility (Table 1). ZnPc is lipophilic and slightly soluble in water or in a commonly-used pharmaceutical vehicle. This hydrophobic characteristics of ZnPc restricts its clinical studies. Cremophor EL is a polyethoxylated castor oil. Its major component is the material in which the hydroxyl groups of the castor oil triglyceride have ethoxylated with ethylene oxide to form polyethylene glycol ethers. This surfactant has been used to enhance solubility of those poorly-soluble materials including Pc by stabilizing emulsions of nonpolar materials in aqueous systems. We utilized Cremophor EL to increase solubility of Photocyanine [23] and ZnPc [24]. The formulation provided us the possibility to investigate in vitro activity and subcellular distribution of Pc analogs in cells.