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REVIEW PAPER

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Chemiluminescence-driven Dye Excitation for Dark Photodynamic Therapy

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ABSTRACT

Photodynamic therapy is a treatment that uses a combination of light-absorbing photosensitizers and dissolved oxygen to kill cancer. One specific limitation of photodynamic therapy is that the visible light used for photosensitizer excitation has a short tissue penetration depth of several millimeters. This limits the application of photodynamic therapy to surface cancers in the absence of a technique to illuminate deeper tissue. Efforts to extend tissue depth to which photodynamic therapy can be applied have been attempted with use of up-conversion and persistent-luminescent nanoparticles that absorb near infrared light and emit visible light for photosensitizer excitation, yet an initial excitation with an external light source is still required. More recently, systems employing chemiluminescence as an excitation energy source designed to bypass the use of external light have been developed and investigated as potential agents that could overcome the problem of achieving photodynamic therapy in deep tissue. We wish to provide an overview of several systems that have been recently reported that employ both radiative and non-radiative chemiluminescent energy transfer for photosensitizer excitation that have been developed in the hope of achieving "dark" photodynamic therapy. This article reviews several of these important new developments in the design of photodynamic therapeutic systems that utilize chemiluminescence.

Keywords. singlet oxygen, chemiluminescence, bioluminescence, photodynamic therapy

Introduction

Photodynamic therapy (PDT) is a cancer treatment that uses photo-generated reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide and hydroxyl radical to damage targeted cells. For generation of ROS, a PDT treatment method employs photosensitizers (PS) that are excited by external illumination provided by visible light at power levels that do not damage healthy tissue. The primary ROS generated is $^1O_2$ which reacts with cell molecules ultimately resulting in tissue damage and cell death. The mechanism of $^1O_2$ formation in these system is energy transfer from excited PS to ground state oxy-

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gen. Other ROS such as superoxide and hydroxyl radical are also generated to a much lesser extent and can be also toxic to cells. Singlet oxygen is short lived (< 1 μs) in biological tissue thus for photodynamic therapy it is desirable to generate $^1O_2$ within a cell. Delivery of PS to diseased tissue is accomplished systemically by injection and PS tends to accumulate on or internalize into targeted cells and in healthy tissue to some extent. After PS delivery to targeted cells, PS is remotely activated by an external light source producing toxic ROS in the presence of oxygen. This methodology imparts spatial and temporal selectivity in treatment as the PS localized on targeted cells is activated by selectively illuminating the region of diseased tissue resulting in necrosis and/or apotosis.[1] Photosensitizers cannot generate ROS in the absence of light or other excitation energy source and ideally PS is nontoxic to heathy cells in the dark. Research in PDT is ongoing as this approach has yet to reach its full clinical potential although several PS are approved for use.[1] Current limitations in PDT include tissue irradiation with visible wavelengths that have a short tissue penetration depth. This particular limitation may be addressed in the design of PDT systems that can excite an acceptor PS by donors via chemiluminescence.

A simplified mechanism for the generation of singlet oxygen is presented in Figure 1. Photosensitizer (PS) excitation from the ground state (^{1}PS) to the first excited state singlet (^{1}PS*) can be accomplished by excitation with external visible light or by chemiluminescence. Intersystem crossing (ISC) to the first excited state triplet (^{3}PS*) followed by collisional energy transfer to ground state oxygen ($^3O_2$) generates cytotoxic singlet oxygen ($^1O_2$) which can induce cell apoptosis and/or necrosis. This review article will address several systems that employ a chemiluminescent donor (chemilumigen) to excite an acceptor PS either by remote illumination or by chemiluminescence resonance energy transfer (CRET). An excellent review article covering mechanisms, structural characteristics of chemilumigens, and applications of chemiluminescence and bioluminescence in PDT has been published in 2016.[2] This review includes several recent developments in the field where a chemilumigen is covalently attached to PS and can excite PS by through-bond energy transfer.

An overview of chemiluminescence-driven PDT systems

One of the first systems developed for overcoming of the need for external light sources in PDT employed the well-known chemilumigen donor luminol. Firer and coworkers investigated the possibility of using luminol as a molecular donor of intracellular chemoluminescence for the destruction of leukemic cells.[3] In this study, murine hybridoma cells were cultured with a transferrin-hematoporphyrin conjugate PS and luminol, hydrogen peroxide and ferrous sulfate and kept in the dark. The results of the study confirmed that the compound luminol induces intracellular chemiluminescence and was able to activate the hematoporphyrin conjugate resulting in 95% cytotoxicity.[3] Although luminol chemiluminescence was sufficient to generate high cytotoxicity by excitation of PS, the concentration of the conjugate PS required to attain LD_{max} was 6 times higher than that needed with an external light source.[3] It is also worth noting that in this study luminol itself induced about 15% cytotoxicity. This study demonstrated the potential for utilizing luminol chemiluminescence as an excitation source in PDT.

Wang and coworkers also proposed a PDT system in which the photosensitizer was activated by a chemiluminescent chemical donor rather than by an external light source.[4] The chemiluminescent molecule, as in the previously described system, was luminol in the presence of hydrogen peroxide and horseradish peroxidase as oxidants. Cationic oligo (p-phenylene vinylene) (OPV) was used as the acceptor PS. Electrostatic binding of the cationic OVP with the negatively charged oxidized luminol dianion placed the donor and acceptor in close proximity for chemiluminescent resonance energy transfer (CRET). Excitation of OVP was achieved as luminol chemiluminescence has a maximum at 425 nm and OVP has an

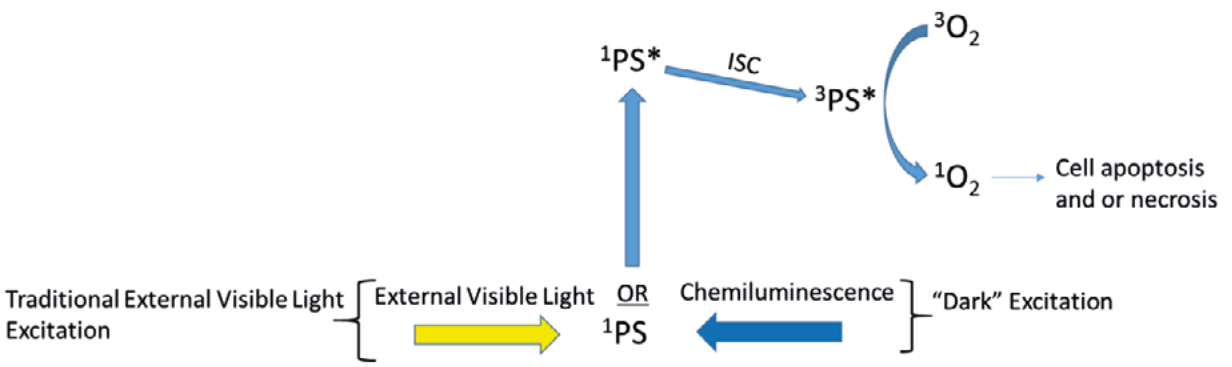

Figure 1. Photosensitizer (PS) excitation from the ground state (^{1}PS) to the 1st excited state singlet (^{1}PS*) by excitation with external visible light or, alternatively, by chemiluminescence. Intersystem crossing (ISC) to the first excited state triplet (^{3}PS*) followed by energy transfer to ground state oxygen ($^3O_2$) generates cytotoxic singlet oxygen ($^1O_2$)

overlapping absorption between 350-550 nm.[4] Oxygen molecules in the surroundings were sensitized through excited OPV to produce reactive oxygen species (ROS) which presumably included $^1O_2$.

Renilla luciferase-immobilized quantum dot-655 (QD-RLuc8) have been employed for bioluminescence resonance energy transfer (BRET) mediated PDT and was tested as an intracellular excitation source.[5] Studies showed that QD-RLuc8 exhibited chemiluminescence at 655 nm after coelenterazine addition for excitation of Foscan® loaded micelles for PDT. This system displayed two separate modes of energy transfer. The first was BRET to the quantum dots. Secondly, the BRET excited quantum dots transferred energy to the PS Foscan® via fluorescence resonance energy transfer (FRET) to yield ROS. Mice tested were transfected with human lung adenocarcinoma epithelial A549 cells and to assess the effectiveness of this system, tumor growth rates were measured in the presence and absence of the QD-RLuc8- coelenterazine-Foscan® system. A significant increase in tumor volume in untreated mice (4.5–6×) compared with mice treated with QD-RLuc8- coelenterazine- Foscan® system indicated delayed tumor growth.[5] In addition, the QD-RLuc8-coelenterazine- Foscan® was also apparently responsible for decreasing vascularization of the tumors. This study showed that the efficiency of bioluminescence-mediated PDT is sufficient for eliciting a photodynamic effect *in vivo* thus the QD-RLuc8- coelenterazine- Foscan® system has the potential to act as a chemiluminescene-driven PDT.[5]

A similar study to evaluate bioluminescence driven PDT were performed by Kim et al. The study was conducted to determine whether bioluminescence is sufficient as an PS excitation source in PDT.[6] To investigate the effect of bioluminescent PDT on tumor growth in mice *in vivo*, the Renilla luciferase-immobilized quantum dot-655 coelenterazine system was activated intracellularly for excitation of the PS chlorin e6. An interesting result was a comparison between the amount of chlorin e6 excitations per minute from a continuous wave laser and from Renilla luciferase-immobilized quantum dot-655 coelenterazine system. Studies determined that chlorin e6 had an excitation rate of 4×10^7 times per minute from a 660 nm 2.2 mW laser and 3×10^8 times per minute from the Renilla luciferase-immobilized quantum dot-655 coelenterazine system This is a very important result showing that bioluminescence resonance energy transfer can generate stronger photochemical activation in the cell membrane than a laser in some cases.[2,6]

The chemiluminescent system developed by Zhang et al. employed functionalized nanoparticles consisting of semiconducting polymer dots (poly[2-methoxy-5-((2-ethylhexyl)oxy)-p-phenylenevinylene) with folic acid and horseradish peroxidase covalently attached on pendant Janus dendrimers.[7] The PS (meta-tetra(hydroxyphenyl)-chlorin) was covalently attached to the core semiconducting polymer dots. Addition of luminol and hydrogen peroxide in the vicinity of these nanoparticle dots was determined to excite PS in two distinct pathways. The first directly by CRET to PS and the secondly CRET to the functionalized nanoparticle dots which in turn excited PS by FRET. This system was tested *in vitro* by incubating the functionalized nanoparticles with MCF-7 breast cancer cells, C6 glioma cells, and NIH 3T3 fibroblast cells. The nanoparticle dots were found to be biocompatible and cell viabilities for fibroblast cells were 72%, glioma cells 32% and breast cancer cells 17% at 10 μg mL.[7] These results demonstrated that the functionalized nanoparticle dot-luminol system was sufficient for chemiluminescent excitation of bound PS for potential use in PDT.

Very recently, Lee et al. have reported the synthesis of "chemi-dynamic nanoparticles" as "dark" PDT systems based on peroxalate chemiluminescence.[8] Peroxalate oxidation by hydrogen peroxide forms an unstable dioxetanedione which decomposes into carbon dioxide and energy which can in turn be used for PS excitation. A hydroxybenzyl alcohol-incorporating copolyoxalate with peroxalate ester linkages and the PS protoporphyrin was evaluated for potential PDT using a cell culture models.[8] The hydroxybenzyl alcohol-incorporating copolyoxalate and protoporphyrin individualy showed minimal apoptotsis in cell models.[8] Interestingly, pre-treatment of cells with hydrogen peroxide-generating cinnamaldehyde followed by addition of chemiluminescent "chemi-dynamic nanoparticles" produced significant dose dependent apoptosis.[8] This study has successfully shown that peroxalate chemiluminescence is sufficient excite PS for cell death in the absence of light.

Chemilumigen donor PS acceptor covalent conjugates

There have been several reported systems that covalently attach a chemilumigen (luminol) to dyes for chemical generation of fluorescence by CRET.[9] Algi et al. studied the photophysical properties and energy transfer efficiency of 2,3-dihydrophthalazine-1,4-dione (a luminol derivative) covalently linked to a boron-dipyrromethene (BODIPY) dye.[9] This conjugate could generate chemiluminescence upon treatment with alkaline hydrogen peroxide in the presence of Fe(III) ions resulting in CRET from 2,3-dihydrophthalazine-1,4-dione to the BODIPY fluorophore. Interestingly, these conjugated donor/acceptor pairs can emit visible light via CRET. These systems can be modified specifically for chemiluminescent driven PDT and, to date, one such system has been developed as depicted in Figure 2.

In this case, in attempts to achieve $^1O_2$ generation, iodine was added to the xanthene core to promote intersystem crossing and the authors observed a significant decrease in fluorescence.[10] Activation of the donor/accep-

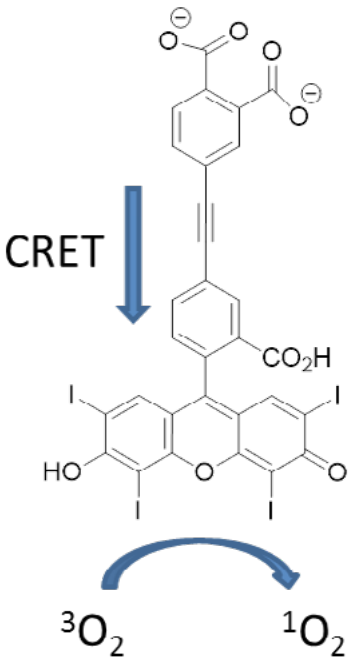

Figure 2. A system developed by Akkaya et al. for excitation of pendant tetraiodoxanthene and subsequent generation of $^1O_2$ via through-bond CRET

tor conjugate with hydrogen peroxide is advantageous since this may also occur readily in cancer cells.[11] Generation of $^1O_2$ was inferred by trapping with 1,3-diphenylisobenzofuran. As far as we know, this is the first system developed utilizing CRET by covalent attachment of a chemilumigen to a PS and further studies are expected to be reported in the near future.

Conclusion

Photodynamic therapy is growing in popularity and usefulness as a clinical treatment for cancer. One main drawback of PDT is that the chemiluminescence driven systems aim to overcome is limited PS excitation light penetration depth. The systems discussed herein are promising as attempts to overcome this limitation although further research and optimization is needed. Given the importance of PDT, we expect that the development of systems that are capable of chemiluminescence driven "dark" excitation of PS will continue to advance.

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