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Antimicrobial photodynamic inactivation: a bright new technique to kill resistant microbes

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Photodynamic therapy (PDT) uses photosensitizers (non-toxic dyes) that are activated by absorption of visible light to form reactive oxygen species (including singlet oxygen) that can oxidize biomolecules and destroy cells. Antimicrobial photodynamic inactivation (aPDI) can treat localized infections. aPDI neither causes any resistance to develop in microbes, nor is affected by existing drug resistance status. We discuss some recent developments in aPDI. New photosensitizers including polycationic conjugates, stable synthetic bacteriochlorins and functionalized fullerenes are described. The microbial killing by aPDI can be synergistically potentiated (several logs) by harmless inorganic salts via photochemistry. Genetically engineered bioluminescent microbial cells allow PDT to treat infections in animal models. Photoantimicrobials have a promising future in the face of the unrelenting increase in antibiotic resistance.

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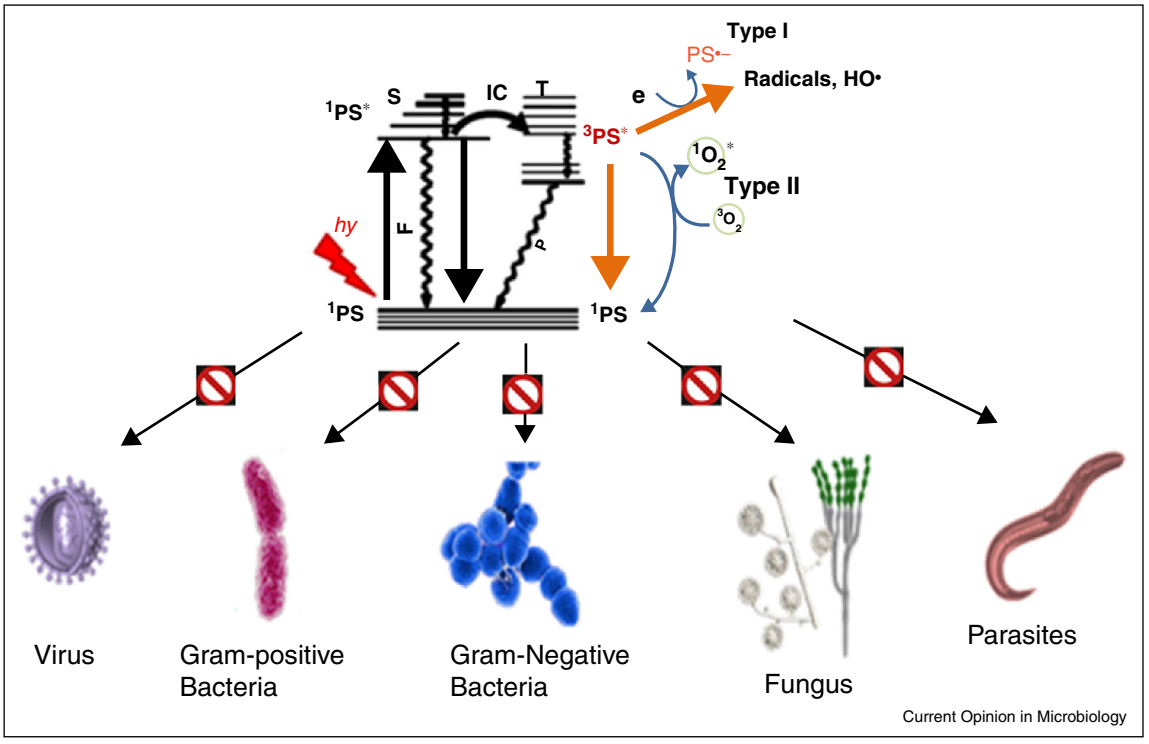
Photodynamic therapy

Photodynamic therapy (PDT) was discovered over one hundred years ago (in the year 1900) by the serendipitous observation that microorganisms (*Paramecia*) were killed when exposed to both a photosensitizing dye (acridine) and sunlight at the same time [1]. However for most of the time since then, PDT has been studied and developed as an anti-cancer therapy, and not as an antimicrobial therapy [2]. The mechanism of action has been investigated in some detail, but still is not completely understood. It involves the absorption of a photon of light (with a wavelength that matches the absorption band of the dye) leading to excitation of the dye (also called a photosensitizer, PS) to its short-lived (nanoseconds) excited singlet electronic state. This singlet-state PS can undergo an electronic transition (spin flip) to a much longer-lived (microseconds) triplet state. The longer lifetime allows the triplet PS to react with ambient (ground state) oxygen by one of two different photochemical pathways, called Type 1 and Type 2. Type 1 involves an electron transfer to produce superoxide radical and then hydroxyl radicals ($HO^{\bullet}$), while Type 2 involves energy transfer to produce excited state singlet oxygen ($^1O_2$). Both $HO^{\bullet}$ and $^1O_2$ are highly reactive oxygen species (ROS) that can damage nearly all types of biomolecules (proteins, lipids and nucleic acids) and kill cells [3]. Figure 1 shows a Jablonski diagram illustrating the photochemical production of different ROS during PDT and their broad-spectrum antimicrobial properties. A Jablonski diagram is a graphical depiction of the PS energy levels in the ground state, excited singlet state and triplet state (top of Figure 1).

Antimicrobial photodynamic inactivation

As mentioned above, for many years PDT was studied as a cancer therapy by designing PS that could be administered either systemically (by intravenous injection) or applied topically (e.g. aminolevulinic acid), and some time later the tumor would be irradiated with light (either as a surface spot or by insertion of interstitial fiber optics) [4]. However starting in the 1990s it was realized that PDT could also exert a powerful antimicrobial effect, if PS could be designed that could selectively bind to microbial cells, while not binding to host mammalian cells [5]. The best way of achieving this goal of antimicrobial photodynamic inactivation (aPDI) was to ensure that the PS had a pronounced cationic charge, as it was realized that microbial cells in general have a more pronounced negative charge compared to mammalian cells and cationic PS will bind selectively. Moreover the binding of the PS to the microbial cells is relatively rapid, while uptake of the cationic PS by mammalian cells is slow, thus giving good selectivity when a short drug-light interval (few minutes) is employed [6]. The advantages of aPDI as a potential clinical antimicrobial therapy were bolstered when it was realized that aPDI works equally well regardless of the antibiotic resistance status of the microbial cells [7] and moreover, that aPDI has (so far) not been shown to produce resistance in bacteria [8] even after 20 successive cycles of partial killing followed by regrowth [9]. Another advantage of aPDI is that the PS is applied topically or locally into the infected area. Many

Figure 1



Jablonski diagram showing photochemical pathways in aPDI. The ground state ^1PS absorbs a photon to form excited singlet state $^1PS^*$ that can undergo intersystem crossing (IC) to form the triplet state $^3PS^*$. This long-lived species can undergo energy transfer (Type II) to form singlet oxygen $^1O_2{}^*$ or elkectron transfer (Type I) to form hydroxyl radicals HO•. Both these ROS are capable of killing a broad spectrum of pathogens.

chronic infections involve a build up of microbial biofilms, into which it is now well-recognized, that systemically administered antibiotics fail to penetrate. However, aPDI has been shown to kill biofilm-grown cells both *in vitro* and *in vivo* [10]. This anti-biofilm application has found particular application in dental infections such as periodontitis [11] and peri-implantitis [12]. Moreover, infections in burns or damaged tissue suffer from a compromised blood supply, so systemically administered antibiotics fail to reach the site of infection in sufficient concentrations. The killing of microbial cells with aPDI is rapid (seconds) while the action of antibiotics can take hours or days, giving a potential advantage against fast-spreading infections such as necrotizing fasciitis. Moreover the broad-spectrum nature of aPDI means that treatment can be instituted before the infectious agents have been identified [13]. Although many infections can occur deep inside the body, it is now possible to deliver both PS and light to almost any anatomical region, via endoscopes and narrow-diameter interstitially inserted needles and fiber optics [14].

New photosensitizers

The optimal molecular design of an antimicrobial PS (aPS) should have several particular features [15]. First of all the aPS should be non-toxic in the dark, especially towards mammalian cells. Secondly they should have good quantum yields of ROS and a high molar absorption coefficient at a wavelength where light penetration of tissue is good (red and near infrared). Thirdly aPS should show selectivity for microbial cells over host mammalian cells particularly at short incubation times (short drug-light interval) [16]. Fourthly and most importantly an aPS should have cationic charges ideally provided by quaternary nitrogen atoms or basic amino groups [17].

Polycationic conjugates

It was previously established that an overall cationic charge was necessary for an efficient antimicrobial PS (especially for one that is required to kill many logs of Gram-negative bacteria) [18]. Therefore it made sense to attach a photochemically efficient PS (such as chlorin(e6) that did not possess any intrinsic cationic charges) to a polycationic polymer that had a large number of them. Proof of principle (*in vitro* and animal studies) was obtained using two broad classes of polycationic polymers, poly-L-lysine (pL-ce6) [19] and polyethylenimine (PEI-ce6) [20] (see Figure 1). The latter compound progressed to a clinical trial in patients suffering from endodontic infections [21] (Figure 2).