Antimicrobial Photodynamic Therapy

Subjects: Others
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Antimicrobial photodynamic therapy (aPDT) has been proposed as an effective alternative method for the adjunctive treatment of all classes of oral infections. The multifactorial nature of its mechanism of action correlates with various influencing factors, involving parameters concerning both the photosensitizer and the light delivery system.

Keywords: aPDT ; dentistry ; laser ; parameters ; PDT ; photodynamic therapy

1. History

The discovery of penicillin by Alexander Fleming in 1928 was one of the scientific highlights of the last century. In the 1940s, antibiotics had been introduced to the market and in the 1980s, pharmaceutical companies were declaring the “end” of infectious diseases. Unfortunately, microorganisms remained, and the extensive and inappropriate use of antibiotics gradually led to the development of pervasive antimicrobial resistance. Since the efficacies of antibiotics decreases and the end of the “antibiotic era” gets closer, efforts to discover new ways to eradicate microorganisms and eliminate multidrug resistance phenomena are evolving. Photodynamic therapy (PDT) therefore serves as a promising approach [1].

2. Photodynamic therapy

Photodynamic therapy is a non-thermal photochemical reaction that involves the excitation of a non-toxic dye (photosensitizer-PS) by light at an appropriate wavelength, to produce a long-lived triplet state that can interact with molecular oxygen to produce reactive oxygen species (ROS), including singlet oxygen ($^1O_2$), which can damage biomolecules, such as polyunsaturated fatty acids [2]. Each of the above-mentioned components (photosensitizer, light and oxygen) are harmless by themselves, but in combination lead to lethal cytotoxic ROS that can selectively destroy cells [3]. This therapy affects the target tissue, which is exposed both to a light source and photosensitizer simultaneously. It shows a dual selectivity, which is based on the different concentrations of the photosensitizer used between normal and target tissue, and also on the spatial confinement of the light only in the target [4].

Photosensitizers are usually organic aromatic molecules with delocalised π electrons, where a central chromophore is covalently bonded to auxiliary substituent branches, which contribute to further electron delocalisation. In this manner, the absorption spectrum of the photosensitizer moiety is modified [5]. They should absorb light at the red or near-infrared wavelengths (600–800 nm). Shorter wavelengths (i.e., those <600 nm) have less penetration and longer wavelengths (i.e., >800 nm) do not have sufficient inherent photonic energy to interact with and induce photodynamic reactions [6].

The source of light must coincide with the absorption maximum of each photosensitizer used. Devices that can be employed include broad-spectrum lamps, light-emitting diodes (LED) or lasers. Amongst these, lasers have specific properties, which render them superior to the other sources. Monochromaticity is a unique and inherent characteristic that provides the laser with the possibility to interact with the photosensitizer by accurately matching its peak absorption. This results in less excess energy and tissue heating, which is sub-optimal in delivering the PDT reaction, when compared to the effects of broad bandwidth devices [7].

The main advantages of PDT are the wide spectrum of antimicrobial action; treatment outcomes are independent of the antibiotic resistance pattern, minimal damage to host tissue, the absence of photo-resistant strains of microorganisms after multiple treatments, a lack of mutagenicity, and minimally invasive and low-cost therapies [8].

Photodynamic therapy has been widely applied for cancer therapy in general medicine. Notwithstanding this, today the interest for antimicrobial PDT has increased in view of the consequences experienced with antibiotic overuse [8]. Several acronyms exist to describe this therapy and in order to avoid any confusion with photodynamic therapy applied for tumour treatments, antimicrobial photodynamic therapy (aPDT) is the most suitable term for antimicrobial purposes [9], as applied in dentistry.

The use of aPDT in dentistry can be readily justified, since the oral cavity is heavily populated with microorganisms, organised within biofilm structures that may show extremely high resistance to conventional antimicrobial agents [1]. Additionally, the uncontrolled systemic use of antibiotics has led to highly resistant microorganisms [10]. Thus, the investigation of an alternative potential treatment for local infections, such as photodynamic therapy, is mandated [11].

3. The mechanism of action of aPDT

The mechanism of action of aPDT can be explained in the following manner: the ground electronic state of the photosensitizer is a singlet state, since it has two electrons paired with opposite spins within its external molecular orbital (highest occupied molecular orbital—HOMO). When the photosensitizer absorbs the appropriate quantum energy from a light source, one of these two electrons is excited to a higher-energy orbital (lowest unoccupied molecular orbital—LUMO). This is termed the first excited singlet-state [12]. To absorb a photon, the energy of the incident photon should be equal or higher than the HOMO–LUMO energy gap and the excess of energy is released through vibrational relaxation; on return to its ground state, the photosensitizer emits the absorbed energy as fluorescence, or produces heat by internal conversion, which is a non-radiative and rapid (less than a nanosecond) process in which electron spins remain the same [8]. Alternatively, the excited singlet-state photosensitizer can undergo a process known as “intersystem crossing” to form a more stable, first excited triplet state. Again, this process is non-radiative and involves a change in spin for the excited electron, so the photosensitizer now has two unpaired but parallel electrons [13]. This endures for <10 ns [8], and the excited triplet state has a lifetime of microseconds [2], so there is sufficient time to induce photochemical reactions. The triplet state also has a lower energy than the excited singlet state [1].

If there is no molecular oxygen (O_2) available, the triplet state photosensitizer can eventually return to the ground state through internal or external fluorescence or phosphorescence [13]. However, in the presence of O_2, the triplet excited state photosensitizer can participate in chemical reactions and provide photodynamic therapy. Indeed, there are two types of these reactions—Type I and Type II [2]. In Type I, hydrogen and electron transfers take place between the triplet excited state of the photosensitizer and other molecules, predominantly O_2. With these chemical reactions, reactive oxygen species (ROS) are produced, that are very active and harmful towards many target cells [13]. These ROS predominantly consist of superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), and singlet oxygen ($^1O_2$) [2]. However, the converse, Type II reaction is much simpler, and involves energy transfer between the triplet state photosensitizer and O_2. This results in the formation of ground state photosensitizer and $^1O_2$ [2].

Singlet oxygen and $^{\bullet}OH$ radical can readily pass through cell membranes and are the most highly reactive ROS species. In view of this, only molecules that are closely located to their site of generation can be affected by photodynamic therapy [6]. Additionally, the lifetime of singlet oxygen ($^1O_2$) is very limited, depending on the surrounding solvent present [14], thus its action radius is approximately 10–55 nm [12]. Hence, the most important factor that influences the outcome of photodynamic therapy is the subcellular localisation of the photosensitizer which drives the process.

In general, the efficiency of the treatment can be affected by the following factors [6]:

- As noted above, the sub-cellular localisation of the photosensitizer. Within the target cell, the photosensitizer may affect lysosomes, mitochondria, the plasma membrane, Golgi apparatus and the endoplasmic reticulum. Most of the photosensitizers localise within mitochondria, where apoptosis is provoked via mitochondrial damage; lysosomes accumulate photosensitizers with more aggregation. The photosensitizer Foscan (a chlorin named *m*-tetrahydroxyphenylchlorin) may target the Golgi apparatus and the endoplasmic reticulum [6]. However, the plasma membrane is rarely noted as a site of photosensitizer accumulation [10].

- The chemical characteristics of the photosensitizer. The different physiology of Gram-positive and Gram-negative bacteria can affect the degree of binding of different photosensitizers. Indeed, Gram-positive bacteria can efficiently bind to cationic, neutral and anionic photosensitizers, while only cationic ones can bind to Gram-negative bacteria [15].

- The concentration of the photosensitizer applied. High concentrations of photosensitizer can be naturally cytotoxic in a non-illuminated state, and obstruct light transmission into tissue target sites [16].

- The blood serum content. The presence of serum in the medium can decrease the effectiveness of the therapy, in view of probable chemical and physicochemical interactions between such agents and selected serum biomolecules [17].

- The incubation time, also known as equilibration time, of the photosensitizer at target sites. This should ideally commence shortly prior to illumination (of a ca. a few minutes’ duration), since this favours localisation into the microorganisms, and does not allow penetration into host cells (this process requires many hours to occur) [18].

- The phenotype of the target cell. It is known that different tissue types have differential light optical properties of light (i.e., absorption and scattering) [6].

An understanding of the mode of action of antimicrobial photodynamic therapy and a knowledge of the structure of the target host tissue is essential. This should facilitate determination of the correct choice of photosensitizer (type, concentration, incubation time, etc.), and the correct light source (kind, power, illumination time, energy, spot size, distance from the target, technique applied, etc.) in order to produce a standardized protocol.

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