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Cancer Treatment Using Multiphoton Photodynamic Therapy

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Abstract

Photodynamic therapy (PDT), a newly established treatment for solid tumors, involves the systemic administration of a tumor localizing photosensitizer that is only activated when exposed to light of appropriate wavelength. Photoactivation of photosensitizer in the presence of oxygen results in the formation of highly cytotoxic molecular species, which precipitates necrosis. PDT has now become a promising means for the treatment of cancer due to its specificity, relatively minimal side effects, and inexpensive. However, the application of PDT has been restricted to the treatment of superficial lesions or the use of interstitial light delivery. A single photon generally activates the photochemical reaction in traditional PDT. However the use of multiphoton excitation, where two or more photons simultaneously excite a photosensitizer, allows for the use of wavelengths twice as long. Such wavelengths exhibit better transmittance through tissue and thereby deeper penetration is achieved. This paper will review theoretical principles of multiphoton excitation, challenges associated with multiphoton PDT and update the current and future role of multiphoton PDT in cancer.

Keywords: Photodynamic therapy (PDT), tumors, photosensitizer, multiphoton excitation

Photodynamic therapy (PDT) is a new approach to treat cancer of various types such as lung, skin, head, neck, throat and reproductive organs. The therapy is based on a photochemical reaction, which is initiated by light irradiation. The three fundamental components of PDT are light, a photosensitizer and oxygen where the photosensitizer is a light sensitive molecule and accumulates to a higher degree in some types of diseased tissue (especially for malignant tumours) than in normal tissue. Following administration of the photosensitizer, its concentration becomes 2-5 times higher in the cancer cells than the surrounding healthy cells. When the photosensitizer is exposed to specific wavelengths of light, it becomes activated from a ground state to an excited state. As it returns to the ground state, it releases energy which is transferred to oxygen to generate reactive oxygen species (ROS), such as singlet oxygen and free radicals which mediate cellular toxicity, leading to local cell death (Fig. 1).

Therefore, PDT allows for selective destruction of tumours while leaving normal tissue intact[5,8,11,12,18,19]. In addition, it avoids the often horrific side effects of cancer chemotherapy and radiation therapy especially, hair loss, weakness, toxicity and harm health cells. However, one of the main limitations of PDT is the low penetration in tissue of the wavelengths used in the treatment. Therefore, PDT is restricted to treatment of superficial lesions or the use of interstitial light delivery through fibre optics. Thus, there has been increasing research in developing photosensitizers at desired wavelengths capable of penetrating deeper into tissue[6,13]. Although most of the photosensitizers are usually activated around 600 nm to 700 nm, there is still a relatively large degree of absorbance and light scattering by various substances present within the tissue, such as water, haemoglobin and melanin, thereby compromising its efficacy[8]. The penetration of light in tissue is however much better for light in the Near-Infrared (NIR) region between 700-1,300 nm (tissue optical window) where the absorption is much lower. The problem is that most molecules have no absorption bands in this wavelength region, and at present, no photosensitiser is available with absorption in this region.

To alleviate such issue of tissue absorption, multiphoton activation may now be a logical choice. In multiphoton activation, the photosensitizers must be excited in a non-linear process, where the energy of two and more photons is combined to excite one molecule. This allows for the use of photons exhibiting only half the required energy for single photon activation. Thus, wavelengths of 1,200 nm to 1,400 nm may be used, which is within the range of tissue optical window. This could greatly introduce the efficacy of PDT application[8].

The recent availability of commercial coherent

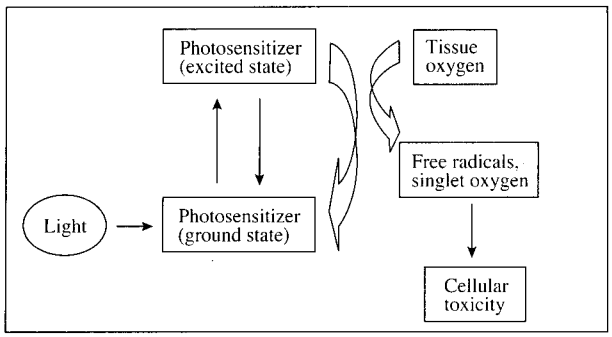

Fig. 1. Mechanism of action of photodynamic therapy[8].

mode-locked lasers and detailed characterization of fibre optics and their effects upon pulsed light has brought multiphoton PDT closer to reality, although there are many engineering issues and a number of new challenges that must be addressed. Thus, this paper will highlight the theoretical principles of multiphoton excitation; challenges associated with multiphoton PDT and future prospective in management of cancer.

History of Photodynamic Therapy

Light had been used to treat a number of diseases since antiquity. However, the advent of modern photodynamic therapy was born in 1900, when a German medical student, Oscar Raab discovered the lethal effects of acridine red and light upon paramecium species. He would eventually discover the antimicrobial effect of acridine was dependent upon specific wavelengths of light and postulated that energy from light was being transferred to the chemical responsible for cell death. At the same year, French neurologists using eosin for the treatment of epilepsy discovered their patients exhibited dermatitis on sun-exposed areas of skin. They eventually used eosin in presence of white light to treat skin tumors thereby demonstrating the first therapeutic application of a photosensitizer. They would also discover the necessity of oxygen for photosensitization reactions and introduced the term "photodynamic action"[1].

In the early nineteenth century, hematoporphyrin was found to act as a photosensitzer after administrating it to mice, whereby skin reactions were observed. In 1913, this was then followed by the first human trial, where a German scientist injected to his own body with 200 mg of hematoporphyrin and observed prolonged pain and swelling in light exposed areas[1]. Localization in malignant tumours was also achieved, when rat sarcomas exhibited the characteristic red fluorescence of hematoprophyrin upon exposure to UV light. Since then an extensive research has been followed in assessing the ability of hematoprophyrin and its derivatives (Porphyrins or HPD) to localize in tumours, as a potential means of tumour diagnosis and treatment[1].

It is reported that Hematoporphyrin may act as a selective photosensitizing agent for the removal of tumors[7]. The mice exhibited a marked reduction in tumour growth after the injection of hematoporphyrin and exposure to light. Tumour growth was suppressed for 10-20 days but eventually, viable areas from deeper regions of the tumours began growing again suggesting insufficient light penetration. After a few years, the first complete removal of a tumour in mice was then reported by Dougherty *et al.*[9] by administering hematoporphyrin derivative (HPD) into mice exhibiting mammary tumours[9]. Around the same time Kelly and Snell[14], reported that light activation of HPD also eliminated bladder carcinoma in mice.

Several human trials have followed since the experiments on mice, and have successfully treated a number of cancers including bladder, skin, lung, gynaecological, brain and rectal cancers. Though there are some side effects (*i.e.* sunburn, erythema, edema, skin necrosis etc), yet these limitations could be controlled through controlling the administration of the photosensitizer, and the duration of light exposures[8]. In 1993, the first PDT drug, consisting of a mixture of porphyrins, was approved in Canada under the trade name of Photofrin. Recently, some other PDT drugs have been approved in other countries such as Levula, Metvix, Foscan, Vertiporfin, Benzvix and Lutex[8]. The chronological development of PDT and its associates has been depicted in Fig. 2.

Multiphoton Photodynamic Therapy

When light propagates through tissue, it can be either absorbed or scattered. These interactions are strongly wavelength dependent[13]. Molecules, absorbing light especially in the visible and near-IR regions, are called chromophores (not photosensitizers). The most important tissue chromophores in the visible and near-IR wavelength regions are haemoglobin, water and melanin which are also responsible to form heat by absorption and thereby cause unwanted cells damage. The absorption of chromophores decreases monotonically with increasing wavelength. A typical absorption spectrum of tissue is shown in Fig. 3.

The absorption coefficient drops by almost two orders of magnitude when it goes from 400 to 700 nm. However, tissue becomes relatively optically transparent and deeper penetration of photons can be attained within 700 and 1,300 nm (tissue optical window)[10]. In addition to the effects of chromophores

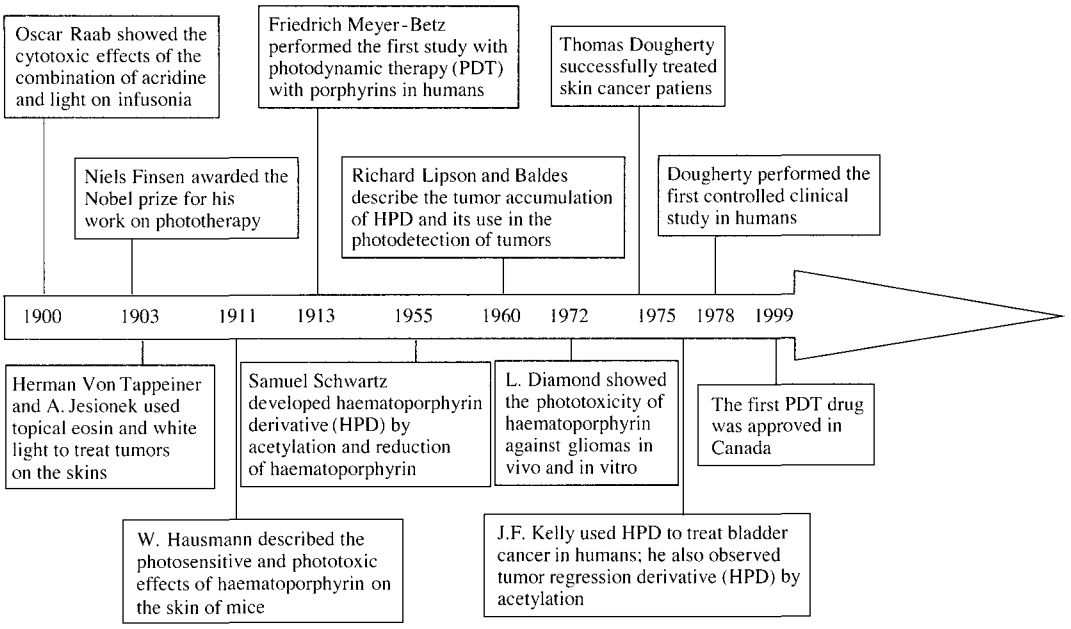

Fig. 2. History of photodynamic therapy (1900-present)[8].

absorption, light scattering is also another limiting factor to the light delivery. Light scattering is inversely proportional to its wavelength for photons in the UV to infrared spectrum. As a result, blue light (380 nm) is scattered considerably more than red light (700 nm). Thus, for the better transmittance of red or infrared light through tissue, it would be desirable to design photosensitizers that undergo its photochemical reaction at such wavelengths. Indeed, many photosensitizers have had its absorption spectra red-shifted to improve the efficacy of light delivery[8]. One of the problems of red-shifting absorption spectra is that it reduces energy transfer from the light to photosensitizer. Therefore, the ability to overcome the activation energy for singlet oxygen formation, which is a severe toxic substance and is thought to activate apoptotic pathways for programmed cell death, is also reduced[1,16].

Rather than developing new photosensitizer, multiphoton excitation has been proposed to provide a possible means of resolving this issue. In multiphoton process, at least two photons combine their energy to excite photosensitizer. The combined energy of the two photons should correspond to that of one photon normally used to excite the molecule, which means that photons with wavelengths in the NIR can be used to excite photosensitizer with absorption bands in the UV and visible region. Thus multiphoton activation would allow for the use of wavelengths twice as long, introducing light delivery within tissue optical window[13]. On the other hand, in comparison to the laser intensity required for single photon activation, there would need to be 30 orders of magnitudes higher for a multiphoton event to produce an equivalent level of activation. Such excessive laser intensities could easily vaporize tissue[13].

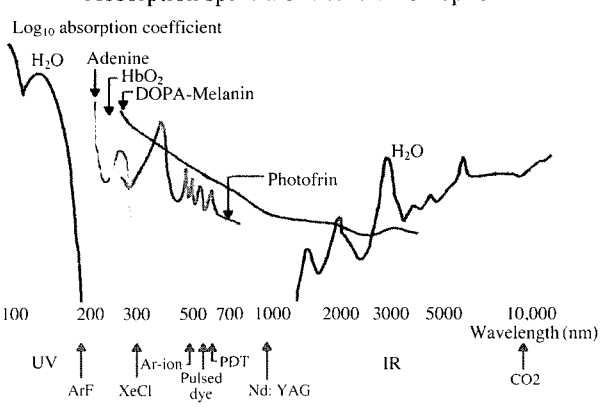

Fig. 3. Absorption spectra of tissue chromophores: An optical window exists between 700 nm to 1,300 nm within tissue[6].

To overcome the damaging effects of such intensities, the use of mode-locked lasers is the common choice. Mode-locked lasers generally consist of a high reflector (*i.e.* a mirror), a gain medium and an output coupler/partial reflector (*i.e.* a one way mirror),

all contained within the laser cavity. Atoms contained within the gain medium are prepared so that light passing through is amplified. The high reflector at one end of the laser cavity and the output coupler at the other end serve to redirect photons back through the gain medium for further amplification. Thus, mode-locked lasers emit exceptionally short, intense pulses (~100 fs) of photons, which are then intensified further by focusing the laser through a lens[15]. However, it is still possible to compromise tissue viability if laser power and pulse width is not properly controlled. As the pulse width broadens, the duration to which the cells are exposed to the intense pulse also increases and compromises their viability. In addition, broadening of the pulse reduces its maximum laser pulse intensity and one would have to increase the power emitted from the laser to produce an equivalent level of photosensitizer activation (Fig. 4)[10].

Thus the effective design of mode-locked lasers results in the production of multiple wavelengths to produce more narrow pulses. In single photon PDT, light delivery is usually achieved through fibre optics which allows for light to be directly transmitted to affected regions within the body cavity. Such an approach however is not feasible for multiphoton PDT. Since it is composed of photons of differing wavelengths, a pulse from a mode-locked laser will broaden when it travel through the fibre optics. Such extensive pulse broadening introduces problems on the use of fibre optics for the mode-locked lasers[2]. Therefore, the optical characteristics of the fibre optics must be carefully characterized to overcome the effects of pulse broadening. When the extent of pulse broadening is quantified properly, the mode-locked laser can be passed through an adjustable autocorrelator prior to entering a fibre optic. An autocorrelator modifies the pulse broadening by broadening the pulse in the opposite direction, leading to recompresses pulse to its original width. This in turn could facilitate the delivery of mode-locked lasers to the specific areas of interest[17].

At present, a number of articles have been published to have a potential role of multiphoton PDT. In 1999, Wachter *et al.* demonstrated that mice exhibiting M-3 melanoma tumors were treated with a melanin precursor derived photosensitiser, DHICA. After exposing focused, mode-locked laser at 1,047 nm on the tumour, the investigators had observed immediate tumour necrosis while surrounding healthy tissue remained viable. Then after 18 days later, the tumour was deemed to be gone completely. In the same time, Konig *et al.*[15] reported that Chinese Hamster Ovary (CHO) cells labelled with Photophrin, were scanned by a focused, mode-locked laser. Upon exposing the cells to 10 mJ/cell, a 50% reduction in cloning efficiency was observed, thereby supporting the potential of multiphoton PDT.

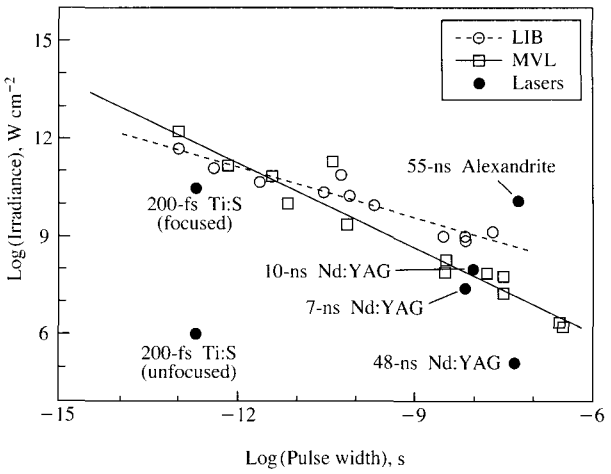

Fig. 4. Effect of laser pulse width upon tissue: Both laser-induced damage threshold (LIB) and minimum visible lesion threshold (MVL) decreases as pulse width increases[10].

Future Challenges

Although many challenges facing multiphoton PDT are being addressed, there are still other issues that must be considered. One of the main issue surrounding multiphoton PDT is the absence of photosensitizers for multiphoton activation. Though existing photosensitizers are capable of multiphoton activation, these photosensitizers have been basically designed for single photon activation. As a result, though their excitation spectra have been red-shifted to improve light penetration, such shifts may result in multiphoton excitation spectra that are red-shifted beyond the tissue optical window. More than 1,300 nm wavelength, the absorption spectrum of water becomes significant once more, and light penetration becomes poor (Fig. 3). Moreover, multiphoton excitation spectra of most existing photosensitizers are still unknown. Thus, it is necessary to search for new photosensitizers especially designed for multiphoton PDT and more thorough characterization must be performed to determine optimal multiphoton wavelengths. Researchers are now also investigating the ability to improve the tumour specificity of existing photosensitizers by conjugating them to tumour associated antibodies. However, this approach arises some problems including complicated synthesis, transport barriers and potential toxicity, thereby its feasibility need to be accurately assessed.

Another issue regarding multiphoton PDT is the

need to focus a mode-locked laser to the affected area results in an exceptionally small volume being illuminated ($\sim 10^{-15}$ L)[4]. To overcome this problem, there might have various effective strategies including scanning the focal point over an area or perhaps using a lens with a higher numerical aperture. The use of large numerical aperture lens could result in larger focal volumes, but it will also eventually reduce the extent of photosensitizer activation. This is because the photon density decreases with the larger volume thereby reducing the probability of a multiphoton event. Using more powerful lasers could compensate this, but tissue viability may be compromised. Though, Laser scanners for multiphoton excitation have been commonly used for multiphoton microscopy, such instruments are quite bulky and need careful design and alignment. This does not readily facilitate its use on patients. The current development of an endoscopic multiphoton microscope could effectively introduce ability to scan affected areas by moving the scanner into position rather than the patient. This device however has yet to be applied to photodynamic therapy[3].

Currently, research is also being directed towards improving existing photosensitizers for improved tumour selectively, with fewer side effects. In addition, researchers are also looking at different types of lasers and other light sources. Moreover, the optical characteristics of tissue are also being investigated to facilitate the development of better light delivery systems, including mode-locked lasers[8]. Only when many of the engineering challenges have been addressed as well as with better localization methods and improved protocols and equipment, the efficacy of multiphoton PDT might be improved and thereby realize its potential role as a major form of cancer treatment.

Discussion

Photodynamic therapy (PDT) is emerging as one of the most promising new cancer therapies in medicine. However, more new photosensitizers need to be approved for PDT through further clinical trials on other photosensitizers. Multiphoton PDT will become as an improved means of PDT when the combine effort of engineers and scientists can resolve a variety of issues that surround its use. When this has been accomplished, comparisons can only then be made between single photon activation and multiphoton activation. Recently, the potential benefits of using multiphoton PDT makes it a promising approach for light delivery, but only with future development and more investigation can its feasibility be accurately assessed. Multiphoton photodynamic therapy could therefore be an important cancer treatment of the future.

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