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Review

# Local treatment of viral disease using photodynamic therapy

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**Abstract**

Although reports of the photodynamic inactivation of viruses appeared in 1928, long before chemotherapeutic antiviral drugs, the first clinical trial in humans—the topical treatment of herpes genitalis—did not take place until the early 1970s. Trials were discontinued due to the transformation of healthy cells and concomitant incidence of Bowen's disease in some patients, probably due to the migration of infective sections of photodamaged viral nucleic acid. With the modern development of photodynamic therapy as a cancer treatment and the use of photosensitisers in the photodecontamination of blood products, a great deal of experience has been gained, both in the minimisation of side effects in humans and in the targeting and eradication of viruses. This suggests that the photodynamic approach to a range of virus-associated infections, lesions and cancer might now be revisited with greater success.



*Keywords:* AIDS; Herpes simplex; Papillomavirus; Photodynamic therapy; Photosensitiser; Virus

## 1. Introduction

The use of dyes to inactivate microbial species has been known since the beginning of the 20th century [1], with the demonstration of the technique against viruses initially reported in 1928 [2]. Clinically, this was first applied to the treatment of herpes infection by Felber et al. in the early 1970s [3] and the protocol enjoyed considerable early success, particularly in the treatment of herpes genitalis in clinics in the United States [3,4]. However, since the mode of action of the photodye used, Neutral Red (Fig. 1) was reportedly oxidative scission of viral nucleic acid, it was argued from an early stage that this might cause side effects in humans, either by the transformation of normal into malignant cells, or by nucleic acid photodamage in uninfected cells surrounding the herpetic lesion [5]. In addition, some clinical trials showed no beneficial effect of Neutral Red against a placebo [6,7]. Following this, the presentation of several patients with post-treatment Bowen's disease of the genital region was sufficient contemporary evidence to discourage the practice [8].

In common with other planar heteroaromatic dyes, such as Proflavine and Methylene Blue (MB) (Fig. 1), the molecular shape of Neutral Red makes nucleic acid intercalation quite straightforward. Damage at the nucleic acid level is normally produced by the attack of photogenerated singlet oxygen, a highly reactive species that causes oxidative degradation, especially of guanine residues [9,10]. Viral photodamage can thus lead to the production of short segments of nucleic acid that might transform healthy cells. Similarly, nucleic acid damage in healthy cells holds the potential for host mutagenicity.

The use of photosensitisers in the treatment of topical viral infection might, therefore seem, at this distance, to be a non-starter. This would certainly be true if the evidence of the US clinical trials were the sole consideration. However, with the modern development of the photodynamic technique, particularly in the treatment of topical cancers, there has been an enormous experiential increase in the practice of human phototreatment, both from the point of view of photosensitiser type and of light delivery [11]. In addition, the non-oncological applications of the photodynamic technique now include a wide-ranging antimicrobial portfolio [12]. Recent activity in the field of blood product disinfection has been based on a photovirucidal approach, with the requirement that the other microbial branches are also

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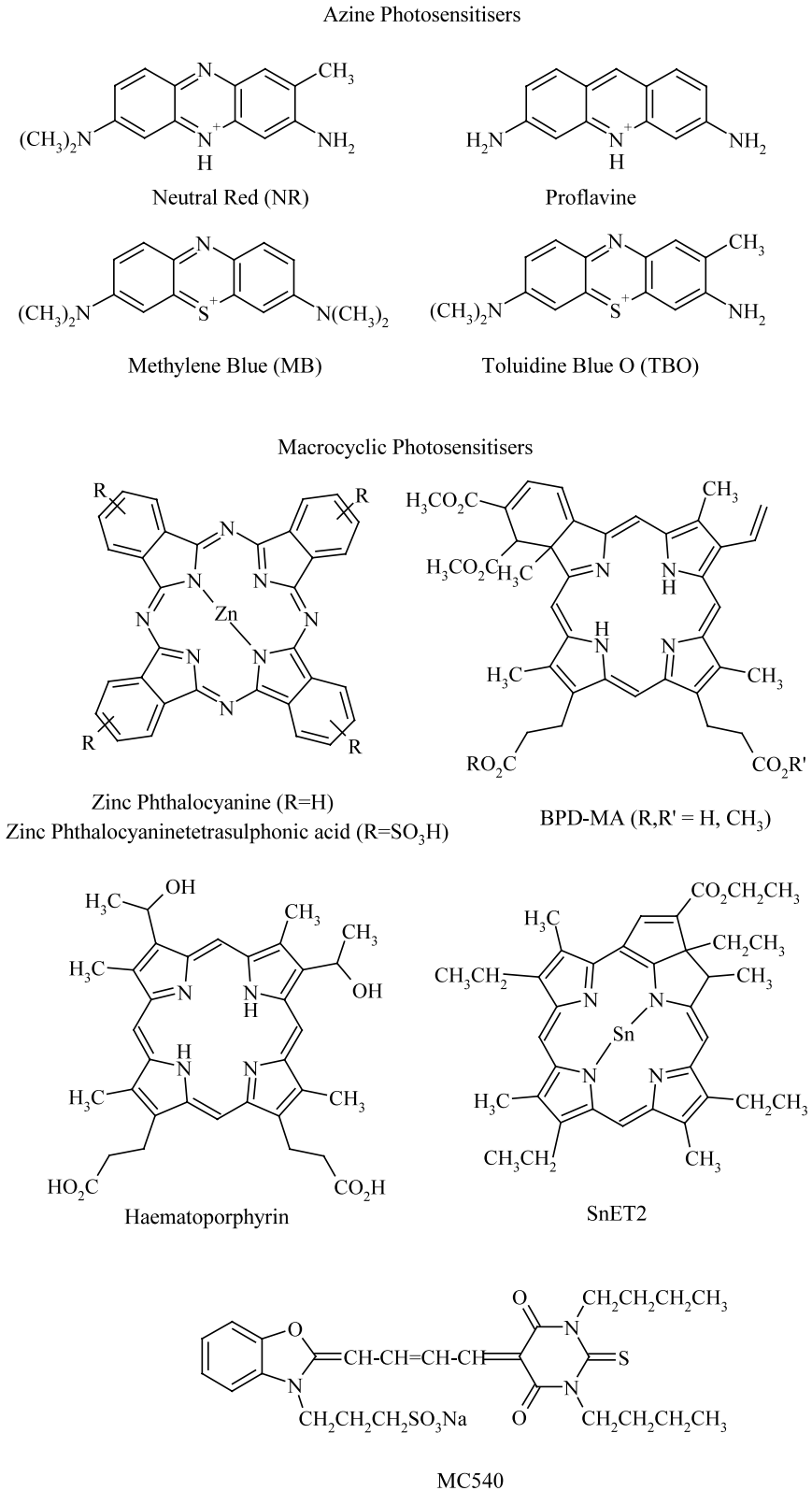


Fig. 1. Chemical structures of the photosensitisers.

susceptible [13]. In terms of medicinal chemistry, the targeting of viruses in blood fractions has mainly concerned viral nucleic acid, thus planar molecules such as MB and the psoralens have again been employed; indeed MB disinfection of plasma is used by several European agencies [14]. Viral targeting in this

way is acceptable in terms of collateral damage since plasma and therapeutic blood cell fractions do not contain nucleic acids.

### 1.1. Viral disease

Herpesvirus-associated disease in *Homo sapiens* has been ever present and chronic, low level infection is common, emphasising the longevity of the relationship between man and virus. Disease of the skin and mucous membranes is typified by herpes labialis (oral), keratitis (ocular) and genitalis, all due to herpes simplex virus (HSV1 & 2), with more extensive lesions present in herpes zoster infection. In each case initial infection is normally sub-acute, but recrudescence is common, especially in herpes simplex infection, and more delicate structures may be permanently damaged, for example in ocular keratitis. Herpesvirus (human herpes virus 8 (HHV8)) is also associated with Kaposi's sarcoma in HIV-positive patients.

Papillomavirus is well known to be associated with the development of cervical cancer [15]. A recent study has suggested that HIV-1-positive women are at increased risk of development of invasive vulvar carcinoma and other malignancies associated with HPV [16]. HPV has also been implicated in oral squamous cell carcinoma [17] and Barrett's oesophagus [18].

Since the idea of a simple, cost effective treatment for topical viral infection is attractive and there is clear evidence that a range of effective photovirucidal agents exists, a properly conducted, thorough trial of such agents might now be carried out.

### 1.2. Current therapies

#### 1.2.1. Herpesvirus infection

Conventional systemic (oral) drug treatments, e.g. for genital herpes, are typically based on nucleotide analogues such as acyclovir, famciclovir and valacyclovir. Such drugs are used to shorten the course and decrease the severity of disease episodes, may suppress the virus itself and prevent future outbreaks of genital herpes [19]. Although the incidence of antiviral drug resistance in the general population is not perceived as a major problem in the same terms as multidrug-resistant bacteria, antiviral resistant HSV is prevalent in the immunocompromised population at rates of up to 14% [20], although a more typical figure of 5% has recently been reported [21]. Thus, there is a greater risk of HSV infection becoming chronic and resistant to therapy in AIDS patients or those undergoing transplant operations or cancer chemotherapy [22,23].

HSV infection of the eye can lead to herpetic stromal keratitis. It has been suggested that the neovascularisation associated with this condition might be a therapeutic target [24]. Interestingly, the treatment of neovasculature in age-related macular degeneration using lipophilic photosensitisers such as BPD-MA (Fig. 1) is now an approved clinical treatment [25].

#### 1.2.2. Papillomavirus infection

Unlike herpesvirus infections, there has been little success in arriving at a specific antiviral therapy against HPV [26] and genital warts and other HPV-associated lesions constitute significant problems in public health. Current therapeutic options include podophyllotoxin and imiquimod cream, a local immunomodulator that induces interferon-α and cytokines in the skin [27,28], or interferon-β gel given as an adjuvant after laser ablation [29]. Other therapies, such as freezing/$CO_2$ laser are administered by the physician. Such approaches may require repeat sessions and can be painful [30]. Trichloroacetic acid (TCA) application in human papillomavirus (HPV) infection and mildly abnormal cervical smears was found to decrease the viral load but not to improve regression of disease [31]. Ablative laser therapy of HPV-associated cancer of the penis has been carried out with excellent outcomes, including cosmesis and function [32].

Systemic strategies against HPV-related cancers include the use of nucleoside analogues such as 5-fluorouracil, antisense oligodeoxynucleotides and vaccines [33,34]. However, the use of PDT against lesions offers both antiviral and antitumour capabilities.

## 2. What has PDT to offer

Photodynamic therapy is a modern approach to the local inactivation of non-economic cells. For the most part, PDT uses established dyes or dye derivatives as the active agent or pro-agent (in the case of singlet oxygen production), and this has not helped the adoption of the technique in the clinical milieu. Additionally, the human toxicology of new photosensitisers is mainly unknown, again hindering clinical trials. Therefore, the introduction of a PDT treatment protocol is difficult, and this difficulty is exacerbated if there is already a conventional therapy available.

Thus, whether it is aimed at the treatment of skin cancer, the disinfection of collected blood plasma fractions or for herpes therapy, the photodynamic approach must offer a clear advantage over what is currently available. In the latter case, PDT does not offer an advantage over proprietary OTC medicines in the treatment of herpes labialis. Cold sores in the general population are routinely managed successfully by self-dosing with the nucleoside analogue acyclovir (Zovirax®). It is hardly to be expected that every home will own a designated light source and dosimeter! Rather, photodynamic treatment in the community is envisaged as being centre-based, either in the outpatient

clinic (dermatology, GU, ophthalmology etc.) or within general practice. Furthermore, treatment would have to be offered only in cases of recalcitrant, drug-resistant or extensive lesions. Advantages might also be offered in the palliation of lesions in immunocompromised patients (e.g. the topical treatment of extensive herpes or Kaposi's sarcoma in AIDS).

### 2.1. Photosensitiser action

Photosensitiser molecules differ from other dye types in that the absorbed light energy can be passed on efficiently to other molecules in the vicinity, or be utilised for photochemical reaction. The electronically excited photosensitiser molecule is relatively stable and can undergo an electronic rearrangement (to the excited triplet state, $^3$Ps*, Fig. 2). Excitational energy transfer from the triplet state to other molecules facilitates photodynamic action. Type I photosensitisation involves direct interaction of the excited triplet state of the photosensitiser with adjacent molecules allowing electron transfer or hydrogen abstraction and the formation of radical species. In the case of psoralens, cycloaddition reactions take place.

Direct transfer of the excitational energy from $^3$Ps* to oxygen causes the formation of a highly reactive species, singlet oxygen (Fig. 2). The formation of highly labile singlet oxygen within a cellular environment leads to non-specific oxidative reactions over a very short time-scale-singlet oxygen will decay to the non-toxic ground state (triplet oxygen) within microseconds.

Whether the photosensitiser is employed in its anticancer or virucidal mode, damage at the molecular level is similar, the gross cytotoxic result being governed by the localisation of the photosensitiser and thus by the differences in cytological make-up between cell types. Type I photodamage due to electron or hydrogen abstraction by the photosensitiser, subsequent redox reactions and oxygenation products rely on close proximity of the photosensitiser and the biomolecular target.

Type I reactions include the abstraction of allylic hydrogens from unsaturated molecules such as phospholipids. Reaction of the radical species thus formed with in situ oxygen leads to lipid hydroperoxide formation. Conversely, aminolipids and/or peptides in the cell envelope may be targeted, leading to the inactivation of enzymes and receptors [35]. Lipid peroxidation has detrimental effects on structural integrity, leading to increased ion permeability (Table 1) [36].

Type II processes are generally accepted as the major pathways in photooxidative viral damage. As with the Type I pathway discussed above, singlet oxygen will also react with molecules involved in the external structure of the cell, such as the aminoacid tryptophan, which undergoes cycloaddition with singlet oxygen, the unstable intermediate product formed degrading to give reactive derivatives which may result in peptide cross-linking. Methionine residues are also oxidised by singlet oxygen (Table 1) [37].

The interaction of tricyclic photosensitisers such as Neutral Red or MB with nucleic acids is well known [10]. Here the site of action is normally at guanosine residues. Again there exists a difference in selectivity between Types I and II processes. The former is mediated through hydroxyl radical attack at the sugar moiety whereas the latter is attack of singlet oxygen at the guanine base [9]. However, and particularly in the current argument if there is low selectivity nucleic acid photodamage can be seen as a double-edged sword.

### 2.2. Photosensitiser types

Photosensitisers have been shown to inactivate a wide range of viruses and from a number of medical angles.

In the search for methods of production of inactivated viruses for vaccines, poliovirus was inactivated at a concentration of $10^{-4}$ M with white light illumination

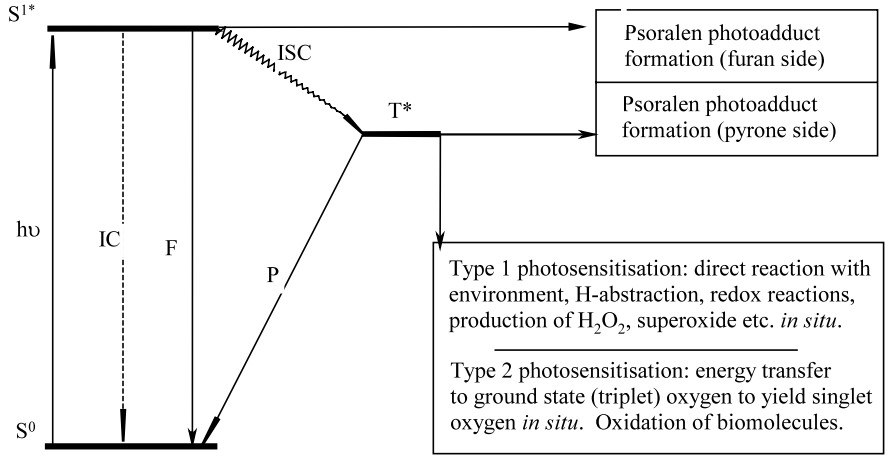


Fig. 2. Photosensitisation pathways.

Table 1
Cellular basis of photovirucidal action

| Site of action | Action | Result | Consequence | Virucidal event |
|---|---|---|---|---|
| Water | Hydrogen abstraction | Formation of hydroxyl radical (HO) | Formation of hydrogen peroxide, superoxide ($O_2^-$) | Further oxidative processes |
| Envelope: unsaturated lipids | Peroxidation | Peroxidation | Hydroperoxide formation | Increased ion permeability ($Na^+$/$K^+$ leakage) |
| Viral protein coat | Hydrogen abstraction | Peptide cross-linking | Enzyme inactivation | Loss of repair facility, lysis |
| Protein coat | Oxidation of Try/Met/His residues | Protein degradation | | Loss of viral infectivity |
| Enzymes (e.g. reverse transcriptase) | Oxidation or cross-linking (as above) | | | Inhibition of ribosome assembly, inhibition of replication/infectivity |
| Nucleic acid residues (typically guanosine) | Oxidation of base or sugar | 8-Hydroxyguanosine formation | Nucleotide degradation Sugar degradation/cleavage | Base substitution, strand cleavage, mutation, inhibition of replication |

Photocytotoxic events at the molecular level.

by several tricyclic photosensitisers including Neutral Red, MB, Toluidine Blue O (Fig. 1) and Proflavine [38]. The procedure was only successful at the highest light dose. Importantly in the case of poliovirus (non-enveloped), mature viruses were not susceptible, the efficacy of the approach being dependent on uptake of the photosensitisers from the medium during viral propagation, the dyes being unable to breach the protein coat [39,40].

The photoinactivation of viruses in blood products is currently a highly active area, and due to the worldwide nature of the industry this must address a large number of viral species. This has focused on the HIV, HSV and hepatitis virus families as the major threat to health via transfusion–transmission. The whole gamut of photosensitiser types has been investigated for use in this area: porphyrins [41], phthalocyanines [42], phenothiazinium dyes [43] and natural product photosensitisers such as hypericin [44] and riboflavin [45], as well as the photoadditive psoralens (see below). Experimental data from this research has promoted understanding of the interactions between photosensitiser molecules and viruses and the way in which this varies both with viral type and photosensitiser structure. For example, the nucleic acid intercalation of heteroaromatic dyes such as MB does not preclude photodamage occurring to other vital structures within the viral particle. Thus, in HIV tests, MB was found to exert a photodynamic effect against the viral core proteins, viral ribonucleic acid and reverse transcriptase [46]. Conversely, in the MB-mediated inactivation of HSV-1, photodamage to viral DNA is reported to be the critical factor [47]. The gathering and assimilation of such information is crucial in the development of structure–activity relationships in the search for safe and effective photosensitisers.

### 2.2.1. Psoralens

Whereas photodynamic action, classified as Type I (redox) or Type II (singlet oxygen-mediated) occurs from the triplet excited state of the photosensitiser (Fig. 2), for photochemotherapeutic reactions, i.e. those typically involving psoralen-type molecules and UVA (300–400 nm), different photochemical products occur depending on whether the reaction occurs at the singlet or triplet stage [48]. Psoralen phototherapy is established in both the treatment of psoriasis (PUVA) and lymphoma. The latter involves the removal of blood and photochemical treatment of the aberrant white cells after separation from other constituents [49].

As with other tricyclic heteroaromatics, psoralens are effective nucleic acid intercalators. Linear furocoumarins (e.g. 8-methoxypsoralen, (MOP) and 4,5′,8-trimethylpsoralen, (TMP) Fig. 3) absorb long-wavelength ultraviolet light, wavebands of 300–400 nm being used for practical illumination. (Absorption of UV light by nucleic acids occurs at lower wavelengths). Intercalated psoralens may thus be excited in situ causing the promotion of [2+2] cycloaddition reactions with olefinic moieties in nucleotide bases, (e.g. cytosine, Fig. 3). The formation of mono-adducts from the furan side of the molecule result exclusively from the singlet excited state, and pyrone-adducts mainly from the triplet (Fig. 2) [50]. Psoralen-type compounds also have the ability to produce singlet oxygen on illumination and photooxidative damage at the site of action may also occur.

Although the typical biological photoreaction of psoralens has been established as cycloaddition with nucleic acid, the furocoumarin nucleus will undergo this reaction with other olefinic moieties, for example in unsaturated fatty acids [51]. The importance of viral nucleic acid targeting by the psoralen can thus be seen in terms of possible side-reactions, e.g. in surrounding tissues.

The major use of psoralen natural products lies in photopheresis (lymphoma treatment) but logically the propensity for nucleic acid binding suggests their use against viral disease. DNA-binding of the lead compound, 8-methoxypsoralen (8-MOP) has been improved upon in newer compounds such as AMT (Fig. 3), due in part to the amino moiety being protonated in the

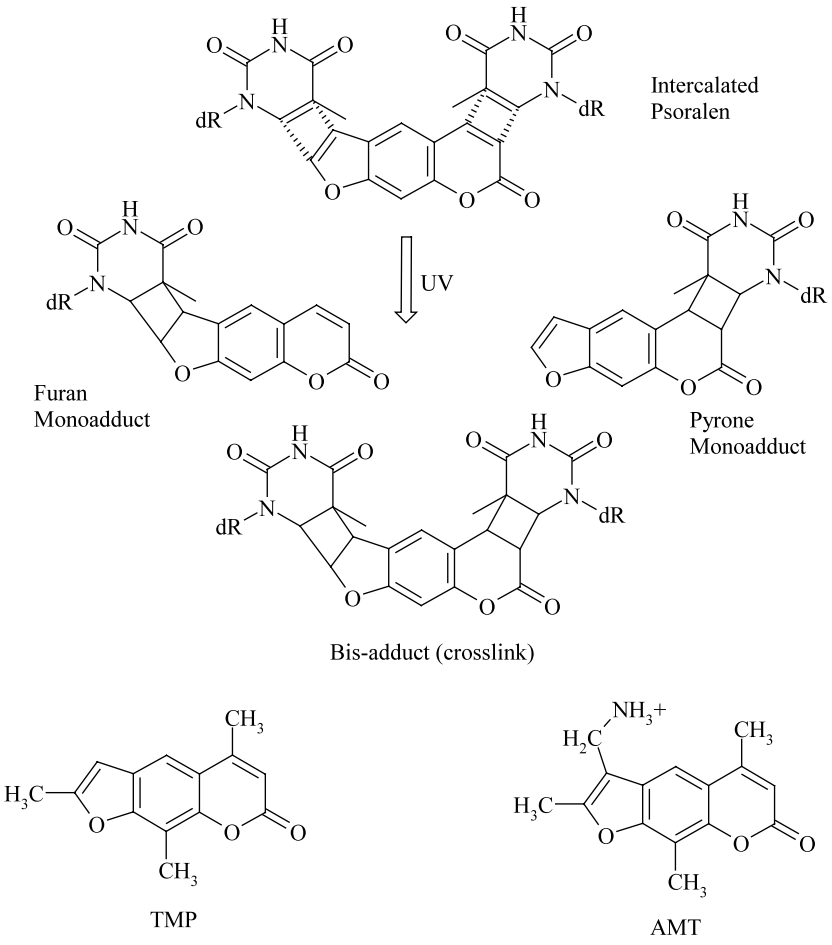


Fig. 3. Photochemical action of psoralens.

physiological pH range [52]. However, the increased binding led to concerns over increased mutagenic potential. In addition, the active spectrum of the psoralens, i.e. below 400 nm may be problematic other than in the treatment of superficial skin lesions due to the minimal depth of tissue penetration of short wavelength light.

With all classes of photosensitiser, the major obstacle to the introduction of PDT into antiviral therapy remains the nightmare scenario of the 1970s herpes genitalis phototreatment. However, there are advantages to be gained by such an approach over conventional protocols and there is now evidence that there are both the photosensitisers and the technological advances required to kill viruses in the clinic without collateral effects in the human host.

## 3. The photodynamic approach

### 3.1. Herpesvirus

Clinical phototreatment of herpesvirus infection with heteroaromatics was widely reported in the 1970s, mainly in the fields of herpes genitalis and ophthalmic keratitis. While this reporting shows that the Neutral Red/Proflavine technique was effective [53,54], indeed it was spoken of as becoming 'the treatment of choice' for herpes genitalis lesser side effects such as contact dermatitis were also seen with clearance of the original infection [55]. Similar effects were noted with Proflavine treatment of idoxuridine-refractory herpes keratitis [56].

The potential for side effects from phototreatment with Neutral Red or Proflavine were acknowledged from an early stage; e.g. fibroblast transformation by Neutral Red-inactivated HSV [57]. However, the combination of impure photosensitisers [58] with white or fluorescent light sources [59] and irregular dosimetry are likely to have increased the risk. Improvement suggestions included that herpetic lesions were painted with Proflavine and that the surfaces were cleaned immediately pre-illumination as well as the use of longer wavelength light to give more effective skin penetration [60].

Other heteroaromatics were also examined in vitro, including Riboflavin (ineffective) and longer wavelength absorbers such as MB [61]. Several analogues of Neutral Red were synthesised and some of their photoproperties

investigated. The ease of analogue preparation suggests that a wide range of derivatives is available, including the possibility of non-intercalating species [62].

Few HSV-inactivation studies have been carried out concentrating on the comparative effects of membrane-active (i.e. envelope targeting) against nucleic acid-active photosensitisers. However, Lytle et al. reported such an investigation using porphyrin esters and zinc phthalocyanine (Fig. 1) versus Proflavine and found that rates of mutation frequency above that of the control cells in vitro occurred only with zinc phthalocyanine of the membrane photosensitisers ( $<2$-fold), and that Proflavine increased the mutation frequency 2–3 fold. In this study the mutation frequency due to UV light was 15–20 times that of the untreated control [63]. It should be emphasised that the illuminating source for the study was broad spectrum visible/near UV light, rather than the more specific, non-mutagenic wavelengths routinely employed in current photodynamic work.

As with mainstream cancer PDT research, activity against viruses in vitro should not be taken as sole evidence of useful activity in vivo. Thus although haematoporphyrin (Fig. 1) was shown to be effective against herpes simplex viruses in Vero cells at concentrations $\geq 5$ mg ml$^{-1}$, no response was elicited in HSV-1-associated keratitis in rabbits [64]. Haematoporphyrin was reportedly inactive against other viruses in culture, including adenoviruses (non-enveloped).

The high phototoxicity of Neutral Red against human fibroblasts led to the suggestion that antiherpetic action was due to general lesion necrosis rather than specific antiviral action, and in conjunction with the much greater concentrations used in human patients was cited as a reason for the side effects experienced in the clinical trials of the dye [65]. Notwithstanding this, VanderWerf et al. have recently suggested the use of Neutral Red as a rational tumour therapy, mainly due to its use in identifying viable tumour cells and its efficacy against human squamous cell carcinoma in vitro [66].

In terms of photovirucidal activity, there are variations in the phenothiazinium series with respect to enveloped and non-enveloped viruses. While it is apparent that MB is less effective against the latter type [67], both MB and Toluidine Blue O have been shown to inactivate the non-enveloped adenoviruses via nucleic acid damage [68,69].

Toluidine Blue O was also shown to be effective in the photokilling of viruses in vitro at the outset of photovirucidal investigation [2]. In early studies by Hiatt et al., TBO exhibited far greater photovirucidal effects against adenoviruses than against polio and coxsackievirus [69].

In testing of Neutral Red, Proflavine and Toluidine Blue O at equal dosage ($10^{-4}$ M) against a range of enveloped and non-enveloped viruses (e.g. HSV, vaccinia, adenovirus, polio and coxsackievirus), TBO completely photoinactivated all but the picornavirus types, whereas Neutral Red and Proflavine were far less effective at this concentration [70].

### 3.2. PDT of papillomavirus infection

Given the activity in herpes infection, there is surprisingly scant evidence of early clinical studies of photodynamic agents, particularly employing the tricyclic photosensitisers used in HSV trials against papillomavirus-associated disease. However, one such trial carried out comparative testing of proflavine and light against hydroxyurea and idoxuridine (idu) in the treatment of papova type warts (mosaic warts on hands/feet). A daylight-type Osram fluorescent tube was used and no light dose was reported (‘6 in for 20 min’). 20% Idoxuridine appeared to give the best treatment, although one case of idu-resistant warts was susceptible to the phototreatment protocol [71].

Perhaps not surprisingly, in recent times, the selective antitumour PDT agent 5-aminolevulinic acid (ALA) has been investigated as a topical treatment for genital warts (condylomata acuminata), having exhibited selective fluorescence in condylomas of the labia minora and vestibule [72]. In a small clinical trial ($n=7$) a 20% (w/w) preparation of ALA was applied to genital warts and the lesions illuminated (usually at 100 J cm$^{-2}$) after 14 h. Although in most cases the lesions were completely eradicated, severe pain associated with illumination represents a significant drawback, and indeed caused two of the subjects to withdraw from the trial [73]. Such problems have been recorded elsewhere in topical ALA treatments [74].

HPV disease of the upper respiratory tract, e.g. laryngeal papillomata, has been treated using PDT, normally employing porphyrin photosensitisers such as haematoporphyrin derivative and dihaematoporphyrin ether [75,76]. Porphyrin PDT, e.g. of laryngeal papillomas, has thus far been palliative rather than curative, and due to intravenous administration of the drug, there are commonly side effects associated with post-treatment skin photosensitisation [77]. Although few serious side effects of PDT have been reported, one patient treated with DHE (Photosan) for laryngeal papillomatosis suffered anaphylaxis post-treatment [78]. However, in a clinical trial of 81 patients with recurrent respiratory papillomatosis using DHE, subjects showed significant improvement, i.e. a significantly larger decrease in papilloma growth rate, at 3 years follow up. Importantly, there was no evidence of photosensitiser induced, virus-related elevation of disease or of carcinoma in situ [79].

The small-scale clinical use of ALA has been reported in viral complications in immunocompromised patients. In this study, ALA was administered in dimethyl sulphoxide with an iron chelator and illumination of the lesions (Molluscum contagiosum and Verrucae

vulgares) was carried out 4 h later. In both cases, the clinical symptoms were greatly diminished after a 1 month follow-up [80]. Previous in vitro studies had shown that the accumulation of PPIX by virally-infected cells to be doubled in the presence of an iron chelator.

### 3.3. Antiviral activity of newer photosensitisers

Generally the efficacy of photosensitising compounds against viruses is governed by few factors.

#### 3.3.1. Virus type

A gross distinction between viruses is often made according to whether the particle is surrounded by a protein sheath or envelope. In the present case, HSV is an enveloped virus, while HPV is non-enveloped. Several photosensitisers have been shown to inactivate both enveloped and non-enveloped viruses, with the former generally being the more susceptible. This behaviour can be explained for planar photosensitisers such as Neutral Red and MB as being due to the ease of their intercalation into viral nucleic acid. The main class of virus inert to these agents is the picornavirus family, the viral particles being very small (e.g. relative to the adenoviruses) and the nucleic acid contained thus being much more compressed. Several early studies on the photoinactivation of poliovirus underline this phenomenon [81,82]. Obviously it is important that photosensitisers are active against all stages of the virus and do not rely on incorporation only by growing viral particles.

#### 3.3.2. Photosensitiser structure

Such elementary properties as molecular charge and relative hydrophobicity are important in determining antiviral activity, as in other areas of drug action. Thus it is more likely that positively charged photosensitisers will be effective in causing nucleic acid damage than will neutral or anionic congeners. For example, each of proflavine, Neutral Red, MB and Toluidine Blue O is a cationic photosensitiser, while the increased activity of the psoralen AMT compared with methoxsalen is reported to be due to the cationic nature of the former [52]. Conversely, anionic phthalocyanine photosensitisers are thought to act against the viral envelope. Thus the membrane-photosensitiser zinc phthalocyaninetetrasulphonic acid (Fig. 1) caused less mutation in HSV than did the DNA-intercalating proflavine, and both treatments were much less mutagenic than UV [83]. Similarly, amphiphilic phthalocyanine derivatives (including a naphthalocyanine) were more effective against cell-associated virus than were hydrophilic analogues. Again photodamage occurred at the viral envelope [84]. Since the major problem in clinical treatment is caused by cell-associated viruses, it is essential that the photosensitiser is able to enter the infected cell before attacking the virus. Here, the influence of drug hydrophobicity is important, and considerable efforts have been made with both anionic and cationic phthalocyanines in this respect [85–87]. Cyanine dyes such as Merocyanine 540 (MC540, Fig. 1) are also known to be membrane active and thus might be considered as non-intercalating photosensitisers [88]. In addition, the membrane-type activity of MC540 is reportedly due to localised heating on photoisomerisation of the excited singlet state [89], thus offering an alternative mode of action.

## 4. The light problem

By definition, photodynamic therapy requires a source of light to supply the requisite energy for singlet oxygen production in situ, or to promote redox reactions (see Fig. 2). The energy required is determined by the molecular structure of the photosensitiser, thus Proflavine has a maximum wavelength of absorption of 456 nm, which is at considerably higher energy than that of Toluidine Blue O (628 nm). The light sources, which were used for photoexcitation in earlier clinical trials employed white or fluorescent light, which contains an element of the ultraviolet region. This is relatively high energy radiation, which can be absorbed by aromatic dyes such as Pf and TBO, but is also absorbed by nucleotide bases and can lead to photochemical breakdown. Mutagenesis by ultraviolet light is well established [90].

In order to remove the possibility of ultraviolet side-effects, more appropriate light sources have been developed for photodynamic therapy, i.e. lasers or pseudo-lasers, filtered light sources etc (Table 2). In terms of photodynamic drug design there now exists a thorough understanding of molecular structure and light absorption characteristics, and a host of long wavelength-absorbing photosensitisers is available (see Table 3).

Light absorption is also important in the treatment of tumours. Since these are likely to extend further than viral lesions, light must be able to penetrate to a deeper level. It has been shown that tissue penetration of light approximately doubles between 500 and 700 nm. In addition, cell pigmentation by definition absorbs light and this must be overcome by any PDT protocol. Thus HHV-8 associated Kaposi's sarcoma—which is a violaceous lesion—is susceptible to PDT and has been shown to respond to treatment with the purpurin SnET2 (Fig. 1, [91]), i.e. long wavelength light is able to circumvent endogenous absorption by the pigmented cells (this has also been shown in melanoma cell lines [92]).

The amount of light given in PDT is highly significant, since this must provide the photoexcitational energy that facilitates subsequent virucidal events, but it is important that the light dose is optimised in order to

Table 2
Light sources available for phototreatment

| | Wavelength (nm) | Power (W) | Power density (mW $cm^{-2}$) |
|---|---|---|---|
| *Lasers* | | | |
| Argon | 488–514 | 20 | |
| Argon ion pumped dye | 585 or 630 | 3–4 | |
| Tuneable dye | 400–1000 | 20 | |
| *Wavelength-filtered lamps* | | | |
| Quartz halogen | 620–640 | 250 | 40 (16 $cm^2$ area) |
| Tungsten filament (slide projector) | >600 | 500 | 7–10 (25 $cm^2$ area) |
| Xenon arc | 600–700 | 1–5 | 150 (20 $cm^2$ area) |

Table 3
Excitation wavelength ranges of photosensitisers

| Photosensitiser type | Wavelength range (nm) |
|---|---|
| Psoralen | 300–400 |
| Acridine | 400–500 |
| Phenazine | 500–550 |
| Cyanine | 500–900 |
| Phenothiazinium | 590–670 |
| Porphyrin | 600–690 |
| Perylenequinonoid | 600–650 |
| Phthalocyanine | 660–700 |

minimise collateral damage to surrounding tissues. This can also be achieved physically by 'blanking' the perilesional area with black tape.

## 5. Conclusions and future prospects

Dermal and epithelial viral infections are ubiquitous. In many cases they constitute a minor inconvenience which can be remedied by the application of standard therapeutic creams or gels, but acute infection suffered by a minority of less fortunate, and often stigmatised, individuals requires a much more involved approach to treatment. Of the two major viruses involved, herpes and papillomavirus, the lesions presented can be treated topically with conventional agents, but with a wide variety of outcomes, exacerbated by the chronic nature of the former type. Although modern chemotherapy of viral disease offers effective short-term relief for HSV infection, recrudescence remains a problem, and there is still no effective treatment for HPV lesions. Thus, in both arenas, PDT may have a valid part to play. The question is: can sufficient proof be provided to allay entrenched fears concerning side effects?

The US trials of 'dye phototherapy' were neither ill-conceived nor poorly carried out. In the early 1970s they represented a logical approach to a worrisome and increasing problem. The materials and techniques employed in the various trials were the best conceivable at the time and we should not condemn at this distance with the benefit of 20 years of cancer PDT development. Had the trials been initiated today significant differences both in the photosensitisers and light sources used would be apparent. Several photosensitisers have already shown promise as antiviral agents, e.g. MB, Toluidine Blue O, Haematoporphyrin derivative and aminolaevulininc acid (PPIX). Each of these exhibits a much safer light absorption profile from the point of view of collateral damage, being activated by long wavelengths (600 nm).

As with the related antitumour mode, the photodynamic therapy of viral disease is only proposed as a topical treatment; light can be delivered effectively to accessible lesions, e.g. of the skin, oral cavity etc., but not systemically for deep-seated disease. We must be satisfied that we can inactivate HIV in donated blood and treat Kaposi's sarcoma using this technique, but we cannot cure AIDS. However, the use of PDT to cure or speedily to palliate lesions caused by herpes or papillomavirus infection does offer an alternative therapy, both for virus-associated lesions and subsequent tumours. This represents a considerable advance on conventional therapies, if we are brave enough to develop it.

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