Dyes, Flies and Sunny Skies: Photodynamic Therapy and Neglected Tropical Diseases

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Abstract

Photodynamic therapy, in its various applications, represents the focused combination of electromagnetic radiation, a chemical – usually a dye - capable of its absorption and conversion, and oxygen to provide cytotoxicity (cell-killing). The effect has been known for over a century and there is considerable clinical use in terms of its application to various cancers. However, the antimicrobial properties of the technology – which are considerable – have received only a lukewarm reception by healthcare providers and the possibilities for tropical disease therapy are mainly unexplored. This is particularly vexatious given both the inexpensive nature of the photosensitisers and light sources available in conjunction with the lack of conventional forward progress in widespread diseases such as leishmaniasis, trypanosomiasis and tuberculosis in the Developing World.

The following review therefore covers the use - or potential use – of the photodynamic approach in this area, mainly with reference to tropical diseases having current 'neglected' status, according to the World Health Organisation.

Keywords: methylene blue; neglected disease; photoantimicrobial; photodynamic therapy; photosensitiser; tropical disease

Introduction

Light–activated or *photo*antimicrobial drugs are not widely appreciated by healthcare organisations in the developed, affluent nations. It is of little surprise, therefore, that the use of these agents in what is termed 'Tropical Medicine' by such nations is mainly unknown. This is a highly ironic situation.

21st Century drug discovery is heavily reliant on *in silico* design, but many of the products of this process still carry the imprint of earlier, experimentally-derived molecules. The earliest drugs, particularly in the field of infection control, were dyes. Indeed it was the efforts of chemists such as Paul Ehrlich in searching for chemical cures for 'tropical' diseases – typically malaria and African trypanosomiasis (sleeping sickness) - which gave birth to rational synthetic drug discovery and chemotherapy [1]. The line of descent can be drawn clearly between drug molecules aimed at various disorders of the central nervous system, such as depression, anxiety or schizophrenia, or those aimed at diseases of warmer climes such as malaria, and early, standard biological dyes and stains. Some of these structural similarities can be seen in Figure 1.

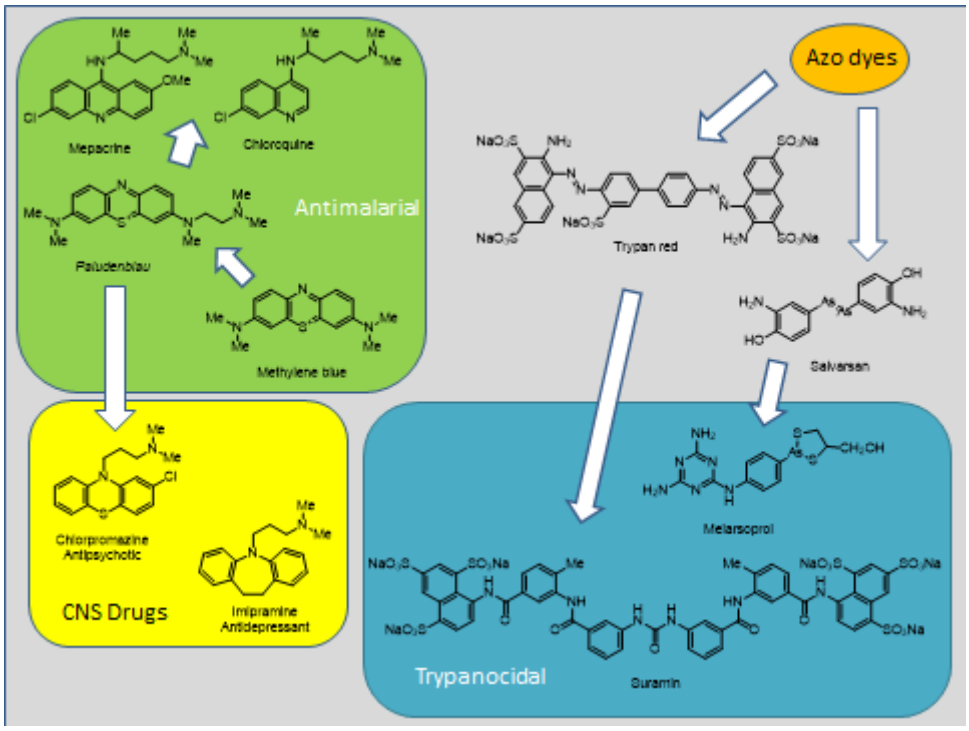


Figure 1. Conventional drugs derived from dyes.

As noted, pioneering scientists such as Ehrlich experimented with the then recently-available aniline dyes to demonstrate selectivity in different cell types, work which led to the demonstration of selective inactivation of certain cells viewed under the microscope. Eventually, in 1891, Ehrlich could report human cures, with two sailors infected with falciparum malaria being cured with the

dye methylene blue [2]. The early antimicrobial (intentionally anti-syphilitic) drug *Salvarsan* – Ehrlich's most famous invention – was derived from experimentation on azoic dyes, the arsenic atoms in the drug which endow its toxicity, replacing the original azoic nitrogens (Figure 1). Ehrlich and his students, of whom there were many, developed what became known as *dye therapy* to cover several tropical diseases and led to the development of what are now thought of as conventional drugs for malaria and African trypanosomiasis, or sleeping sickness (e.g. chloroquine, Suramin and melarsoprol, respectively, Figure 1) [3].

Once effective colourless drug molecules had been introduced, tissue colouration caused by the use of dyes became an undesirable side-effect. Consequently use of dye-therapy then diminished. The introduction of more conventional antimicrobial drugs, such as the penicillins, further diminished the use of dyes, and by the mid-20th century most were considered obsolete.

Given the amount of experimentation concerning therapeutic dyes around the turn of the previous century, it is not surprising that other scientific directions were also followed. Thus it was discovered that dyes could be useful as indicators of the physical environment (pH, oxidation-reduction) and of particular analytes – the presence of specific metal ions, for example [4].

In 1900, Oskar Raab was a student working for two supervisors in Munich, von Tappeiner and Jesionek. In his experiments on unicellular organisms, Raab noted that the synthetic dyes he was using –acridine and several xanthene dyes - caused one species, *Paramecium caudatum*, to stop moving when exposed to light. This stillness was later shown to be cell death and the observation was the first reported example of what was subsequently termed *Photodynamy* [5]. It should be noted that Raab's supervisors were later to take these findings and demonstrate a similar effect against animal tumours [6]. The occurrence of reports of the photodynamic effect was sporadic over the next sixty years, with a significant rise in activity in response to drug-resistant, mainly bacterial, infections in the healthcare sector during the early 1990s.

In terms of tropical diseases of *Homo sapiens*, the greatest contribution of dye therapy has been the provision of antimalarials. Due to the Japanese occupation of the Dutch East Indies from 1942, Allied forces in the South-East Asian theatre required alternatives to quinine, and this was supplied first by the aminoacridine mepacrine and later by the closely related 4-aminoquinoline analogue chloroquine (Figure 1) [7]. Members of the armed forces taking the acridine dye derivative mepacrine often presented a jaundiced appearance due to the persistence of the bright yellow acridine drug in the skin [8]. Chloroquine is a colourless compound and is thus – apparently - non-staining. However, chloroquine photosensitivity is a well-established side-effect of the drug, due to the skin absorption of ultraviolet wavelengths in sunlight [9].

As can be seen from Figure 1, the genealogy of modern antimalarials may be traced back to Ehrlich's use of methylene blue in the late 19th Century. Improved derivatives of the original phenothiazine dye were synthesised by chemists at IG Farben in the inter-war period, having basic side chains (e.g. *Paludenblau*, Figure 1). However, the staining effects were not avoided and this was one of the reasons that the search moved on to acridines and quinolines [10]. Perhaps surprisingly, methylene blue itself is again being used as a conventional antimalarial for the treatment of juvenile disease where there is chloroquine resistance, for example in sub-Saharan Africa, since 2005 [11]. Of course, the disadvantage of staining the patient becomes less important in areas with high rates of childhood mortality and with the continued efficacy of the dye.

Thus, while the conventional treatment of some tropical diseases was supplied initially by dyes, very few now remain in use. However, the established selectivity of a range of dye molecules for various organisms implicated in these neglected diseases suggests a firm basis for the development of a different therapeutic approach, utilising this selectivity and another dye property, that of *photosensitisation*.

Photosensitisers and the photodynamic effect.

Raab's initial discovery involving the inactivation of paramecia occurred when he employed the xanthene dyes (among others) with illumination. Had he used the xanthene dye fluorescein, there would have been no killing effect. Photosensitisers are a subset of dyes and, as this example shows, even closely structurally-related compounds may differ significantly in behaviour.

Photosensitisation in organic dyes relies on their absorption of light energy and their ability to utilise this in chemical or physical reaction – either in the transfer of an electron (Type I photosensitisation) or energy (Type II photosensitisation) to oxygen molecules in close proximity, to produce reactive oxygen species, such as singlet oxygen, the hydroxyl radical and superoxide (Figure 2). Deactivation pathways exist, such as fluorescence – i.e. the release of the energy (or most of it) by the excited-state dye in a single emission – which is what would have been observed by Raab had he used fluorescein.

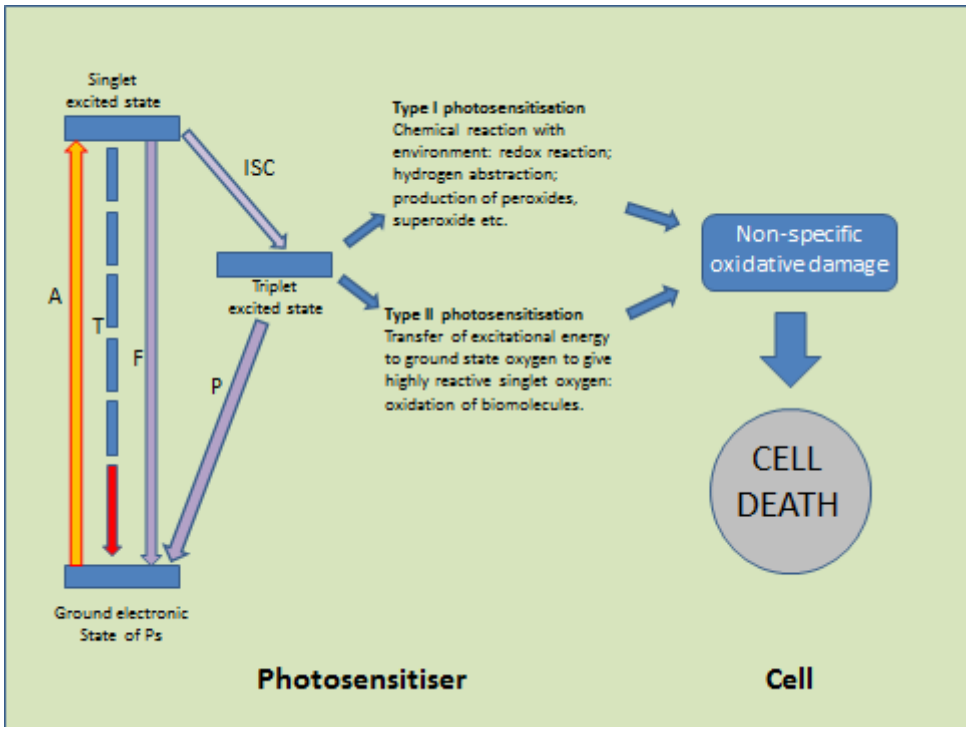
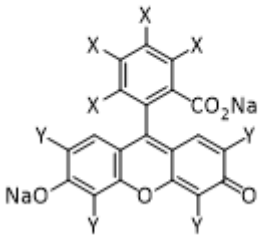
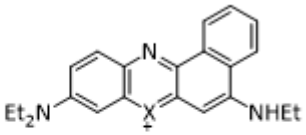


Figure 2. Photosensitisation pathways. Key A – absorption of light; T – thermal deactivation of the singlet excited state; F – fluorescence deactivation; P – phosphorescence deactivation of the triplet state; ISC – inter-system crossing.

The difference in behaviour here lies in the extra stability of the excited-state molecule - e.g. eosin Y, Figure 3 - stabilised, in fact, by the four bromine atoms - fluorescein has none - attached to the chromophore. This is known as the *Heavy Atom Effect* and similar behaviour can be seen in other xanthene photosensitisers erythrosine (four iodine atoms) and rose Bengal (four iodine atoms and four chlorine atoms, Figure 3). Similar effects are seen in the phenoxazine and phenothiazine (or phenoselenazine) dyes, the former type being photodynamically inactive whereas the other classes, containing lower period sulphur or selenium atoms are generally very efficient photosensitisers [12].

	X	Y	$^1O_2$
Fluorescein	H	H	0.03
Eosin Y	H	Br	0.50
Erythrosine	H	I	0.63
Rose Bengal	Cl	I	0.75





X	$^1O_2$
O	0.003
S	0.03
Se	0.78

Figure 3. Photosensitisers and the Heavy Atom Effect. Key: $^1O_2$ – efficiency of singlet oxygen production [13,14].

On illumination with the correct wavelength radiation, photosensitisers are thus able to interact to a far greater extent with their environment than do other dyes, and if this illumination occurs when the molecule is either inside or closely associated with a cell, the ensuing chemical reaction (or reactions) can produce cell damage or death, particularly in simple cells (Figure 2). This was the process first reported by Raab for paramecia.

What was not apparent at the time of Raab's discovery was that the now established therapeutic dye methylene blue is also a photosensitiser, normally working via the Type II pathway and producing the highly reactive molecule singlet oxygen [15]. This is significantly toxic to simple microbial cells. Other standard microbiological dyes which are also photosensitisers include crystal violet, acriflavine and neutral red [16].

Consequently, what became apparent from the sporadic literature output concerning photosensitisers during the early-mid 20th Century was that much of the pathogenic threat to humans and their animals could be very simply disarmed without conventional drugs, at least in the laboratory.

In order to produce cell killing/inactivation by this method there must be interaction of the target cell, photosensitiser and light. In the laboratory setting this is straightforward, but is obviously a somewhat more complex process when the target is inside an individual host. For this reason the clinical application of photosensitisers is, at least at present, confined to topical or local infection. The use of endoscopy/fibre optics allows light delivery to remote sites, with the loci of infection being identified and tracked via ultrasound and X-ray. Infected sites on the body's external surfaces or shallow orificial sites are obviously far more accessible and can be treated superficially.

There is thus an excellent basis for the application of the photodynamic approach to the cure of infectious disease. The requisite conversion *from bench to bedside* has not proved so simple, however, and usually for reasons other than robust scientific rationale.

Tropical Disease and *Neglected* Status

As noted above, the term *tropical disease* is one employed mainly by those resident outside the countries located around the central band of the globe. However, for many years the term also contained a significant implication of the inability of such countries to deal with diseases such as malaria or trypanosomiasis. Given that many of these stricken countries were also normally unable to support a western-style system of healthcare, they were equally incapable of paying the enormous drug fees demanded by large pharmaceutical concerns. Consequently, research into tropical medicines decreased, in many cases ceasing altogether. Interest was somewhat rekindled initially by the human immunodeficiency virus – acquired immunodeficiency syndrome (HIV-AIDS) pandemic, and then when it became apparent that increasing globalisation was promoting the emergence of tropical disease outside the tropics. In both cases, the excellent medical practice of widespread organised blood collection for therapeutic use, suddenly became recognised as a conduit for previously unencountered or, certainly in the case of HIV-AIDS, unknown diseases [17].

The *Drugs for Neglected Diseases initiative* (DNDi) began in 2003, recognising the need with particular reference to African and American trypanomiasis, leishmaniasis, malaria, paediatric HIV and helminth (worm) infections such as schistosomiasis [18]. However, the World Health Organisation's list of neglected tropical diseases is considerably longer, and seventeen of these have been prioritised (Table 1). It will be noted that the WHO list does not include malaria, tuberculosis or HIV-AIDS.

Disease	Causative Organism	Type	Route
Buruli ulcer	*Mycobacterium ulcerans*	Bacterium (Acid Fast)	Aquatic bugs
Chagas' disease	*Trypanosoma cruzi*	Protozoan	Reduviid bug bite
Chikungunya	Virus	Enveloped virus	Mosquito bite
Dengue	Virus	Enveloped virus	Mosquito bite
Dranunculiasis	Guinea worm	Worm	Water flea bite
Echinococcosis	*Echinococcus* spp.	Tapeworm	Animal faeces
Hansen's Disease (Leprosy)	*Mycobacterium leprae*	Bacterium (Acid-Fast)	Infected aerosol
Human African Trypanosomiasis	*Trypanosoma* spp.	Protozoan	Tsetse fly bite
Leishmaniasis	*Leishmania* spp.	Protozoan	Sandfly bite
Lymphatic filariasis (Elephantiasis)	Roundworm, e.g. *Wucheria bancrofti*	Worm	Mosquito bite
Onchocerciasis (River Blindness)	parasitic worm	Worm	Black fly
Rabies	Lyssavirus	Enveloped virus	Animal bite
Schistosomiasis	*S. haematobium* flatworm	Worm	infected water (via urinary tract)
Soil-transmitted helminthiases	Helminths	Worm	Faecal
Trachoma	*Chlamydia trachomatis*	Bacterium (Gram-negative)	Physical contact
Trematodiasis	Flatworms	Worm	Molluscs (food)
Taeniasis/Cysticercosis	Tapeworms	Worm	Food
Yaws	*Treponema pallidum pertenue*	Bacterium (Gram-negative)	Skin contact

Table 1. Alphabetical WHO list of neglected tropical diseases [19]

Infection and Transmission

Most people – in the northern hemisphere, for example - thinking of tropical disease normally imagine insect bites as the cause. This may be some way from the truth, especially given that many such diseases involve the life cycle of a parasite, the incidental bite occurring at some time during this cycle. However, in addition, some diseases are transmitted directly between humans, or between humans and animals, neither requiring the involvement of an insect. Others are caused directly by nematodes or worms. Furthermore, the causative organisms in the various diseases considered here might be bacteria, viruses or protozoa. Such is the overwhelming publicity accorded to malaria, that mosquito bites and plasmodia (the causative protozoal class) dominate the public consciousness as far as this field is concerned.

As has been noted, the photodynamic approach requires the combination of photosensitiser, oxygen and light to produce the desired cell killing effect. From a therapeutic point of view, this is somewhat more complex than conventional drug administration (i.e. tablet or injectable) and has a

significant impact on the type of infection which might be treated photodynamically. Thus, for example, malaria is a blood-borne disease and is treated with systemic drugs - i.e. affecting the whole body - which fight the parasite in the bloodstream (some also target the liver stage of the disease). Clearly it would be difficult to illuminate the whole of the circulatory system, so photodynamic attack in this respect is not currently possible in the clinic. However, other diseases have a localised tissue stage, often in the region of the original bite etc., and this constitutes an ideal presentation for the local application of photosensitiser and light.

Given that most pathogenic organisms appear in the bloodstream during disease - whether tropical or otherwise – the use of donated blood and products derived from this should obviously be avoided if collected during the period of illness, but this should also be the case in the run-up to illness, when the individual is asymptomatic. While this may seem straightforward for individuals who are diseased, it is far less so in the pre-symptomatic period. Furthermore, there are those who will donate blood in the full knowledge that they have an infection – although this is more often the case where payments are made by the collecting agency in order to encourage donation.

Finally, there is the phenomenon of the emerging disease – i.e. infection caused by hitherto unknown, and therefore unlooked-for, pathogens being introduced into the blood supply. Certainly this was the case in the 1980s/early 90s with what is now called the human immunodeficiency virus (HIV) [18]. Far more stringent examination and monitoring of the blood supply – haemovigilance - has resulted [20].

Photosensitising dyes have an important part to play in the protection of the blood supply, including that of blood products, such as the clotting factors used in the treatment of haemophilia. The photodynamic effect can be carried out on isolated blood fractions, such as plasma or platelets [21], and this is covered in the various disease sections below. Clearly, this is a somewhat more indirect approach to the problem of tropical disease, not being an attack on the disease syndrome itself, but rather on its transmission. However, it is no less important for that and, as part of the development of more integrated, better-funded and organised blood collection in many of the countries where such diseases are endemic, it would go a long way towards significantly decreasing the resulting morbidity and mortality.

Identifying Potential Approaches

Table 1 provides information on aetiology for the various diseases listed. Given that the photodynamic approach to infectious disease depends on the presentation of the target organism, these aetiologies can inform the type of application and when this might be employed during the life cycle of that particular microbe. However, it should be remembered – unsurprisingly, since photoantimicrobial research is incredibly poorly funded in richer, developed countries – that there is often little hard evidence for a positive effect and much of this can therefore only be provided by extrapolation.

In terms of attack, there are three, basic modalities: direct application to the infected host (topical/local); photodynamic disinfection of blood products containing the target organism; photodynamic disinfection of waterborne targets (environmental approach). A simple decision tree

is shown in Figure 4. A further consideration here must also be that the use of the photodynamic approach endows some benefit over the *status quo*. This may be in overcoming conventional resistance mechanisms, conserving conventional drugs to avoid resistance development or providing a less damaging alternative (for example, natural photosensitisers *versus* chlorine in water disinfection).

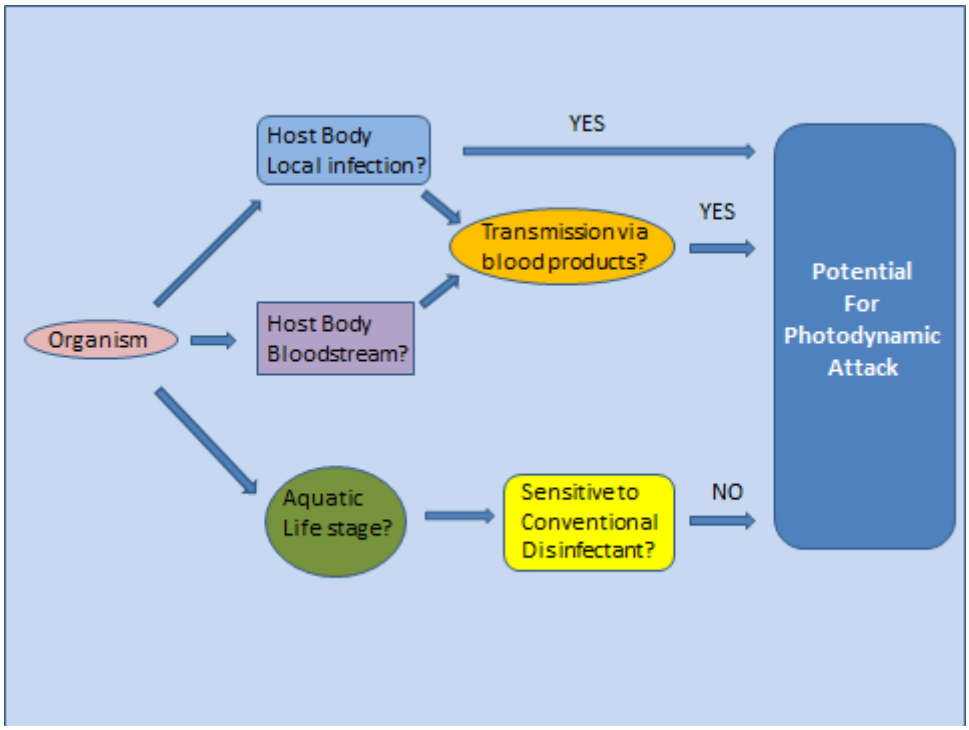


Figure 4. Decision tree to help determine if the photodynamic approach is advantageous.

Clearly, it is not the case that photosensitisers are *the* answer to tropical disease, but there is considerable potential in a number of presentations, as noted in the sections below. A similar situation pertains in infection control in developed countries, but with little uptake currently on the part of healthcare providers [22]. While this does not augur well for the present argument, it may be that medical positions here are rather less well entrenched in the light of neglected status.

The following sections deal with the potential use of photosensitisers either directly to the host, in the treatment of blood/tissue products or of colonised water.

Topical/Local Therapy

Acceptable photosensitisers

The therapeutic application of xenobiotic ('foreign') substances to the host organism should demonstrate toxicity to the target(s) but not the host. While this is rarely possible – there are usually side effects, but these are mostly negligible in commonly-used medications – there is an onus on the developer of the therapy to demonstrate minimal host damage. Consequently, in the early 21st Century, there are very few photosensitisers which are licensed for use in humans or animals [23]. In many ways, this has been the main frustration for groups involved in photosensitising drug discovery over the past quarter century or, to put it another way, the costs involved in satisfying toxicity criteria for new compounds are unattractive to most funding agencies. Consequently, the strength of legislative bodies is such that there is little option but to make initial essays with acceptable photosensitisers to provide a baseline, safe therapeutic approach and on these grounds to seek support for improved photosensitiser development [24].

Currently, the only photoantimicrobial dyes licensed for clinical use – i.e. with light activation - are the phenothiazinium derivatives methylene blue and toluidine blue and the heptacyanine dye indocyanine green [25]. Many others have been synthesised based on both of these dyes classes, as well as others, but little has resulted in terms of movement towards the clinic. There are further dye class examples which have seen previous conventional use as antimicrobials, such as the triphenylmethanes and aminoacridines, as well as food dyes [23]. These have the potential for re-examination in the search for candidates with sufficient photoactivity and lack of host toxicity. Similarly, there are naturally-occurring photosensitisers, such as riboflavin (vitamin B2) and the perylenequinonoid hypericin, from the plant *Hypericum perfloratum* (St John's Wort) [26].

Approaches

As mentioned above, there are various presentations (i.e. the combinations of symptoms/appearance indicating the disease) within the field of tropical diseases where the target organisms are localised to a relatively small volume, rather than being systemically spread. Similarly, others may be more extensive or diffuse but located on the host exterior (skin/hide). Potentially, these presentation are amenable to the photodynamic approach, since both the photosensitising dye and activating light can be applied directly to the site of infection, internal disease being targeted via direct photosensitiser instillation and illumination via fibre optics.

Bacterial infection

Since much of the drive for the inclusion of photoantimicrobials in infection control has come from (usually) academic researchers in the developed world, it is not surprising that there is a considerable literature covering photobactericides, particularly screened against conventionally-resistant hospital bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) [27]. Following 25 years of research in this area the range of susceptible bacteria is wide, covering both Gram types, as would be expected, given the non-selective oxidation caused at the target. However,

in terms of the available dyes, cationic nature is essential for broad-spectrum activity, since anionic and neutral dyes are less active against Gram-negative bacteria, due to the negatively-charged outer membrane of this class [28]. It should be remembered, of course, that the bacteria mentioned also constitute a threat to health in less-developed countries.

Of the tropical diseases identified as bacterial in Table 1, two are Gram-negative (*Chlamydia trachomatis* and *Treponema pallidum pertenue*) while two are *myco*bacterial (*Mycobacterium ulcerans* and *M. leprae*), and are therefore not stained by the Gram system. Although tuberculosis does not have neglected status, it also has a mycobacterial aetiology (*M. tuberculosis*). While Gram-negative bacteria are susceptible to cationic photosensitisers, far less research has been carried out on mycobacteria [29].

Yaws is caused by the entry into the skin, via a cut or abrasion, of the bacterium *T. pallidum pertenue*, which can lead to tissue ulceration or bone infection of left untreated. Current therapy utilises long-acting penicillins or other standard antibacterials [30]. However, there is certainly an argument against conventional drug use, where available, due to the possibility of drug resistance development. Direct application to the original wound using a cationic photoantimicrobial, such as methylene blue or toluidine blue and superficial/interstitial illumination with red light should be sufficient to eradicate the causative organism. The combination of methylene blue and red light has also been shown to cure bone infection (osteomyelitis) [31].

Infection of the eye by *Chlamydia trachomatis*, normally referred to as trachoma, causes damage to the underside of the eyelid. Untreated, this can lead to greater eye damage and, ultimately, blindness. Standard treatment is, again, with conventional antibacterials such as a tetracycline [32]. Localised infection beneath the eyelid might seem a difficult presentation for the photodynamic approach; however, the thinness of the eyelid means that it should be transparent to red light. Consequently, instillation of methylene blue or toluidine blue into the eye with a short incubation time should allow uptake by the chlamydia, and this would be followed by superficial illumination.

It should be noted that both *T. pallidum* and *C. trachomatis* are known to be selectively stained by methylene blue [33,34].

In terms of mycobacteria, the absence of Gram-staining capability might suggest weaker potential photosensitiser interaction, but this is not the case. While the exterior of mycobacteria is made up of long-chain alkanoic acids (mycolic acids), this still constitutes a negatively-charged region to which cations will, logically, bind. It has been shown that each of *M. smegmatis in vitro* and *M. fortuitum*, *M. marinum in vivo* are photoinactivated using methylene blue [35-37]. This also provides an excellent basis for the investigation of its activity against *M. tuberculosis*, and the presence of multiply drug-resistant (MDR) strains should provide little defence against reactive oxygen species (ROS), and singlet oxygen in particular [29]. It is expected that the photosensitiser would be administered using a bronchoscope, while illumination would be either bronchoscopic or superficial, depending on the site of the lesion inside the lung. Conversely, although Hansen's disease (leprosy) has been known as a devastating human disease for thousands of years, the site of the infecting *M. leprae* as a locus of disease is still not firmly established. Its presence in the peripheral nerves, leading to lack of sensation in, and eventual damage, infection and loss of, the extremities, would represent a considerable challenge to the photodynamic approach.

Protozoal infection

In terms of localised infection, the targets here with most potential are cutaneous leishmaniasis and Chagas' disease, both offering relatively easily accessible lesions, and both having parasites which are susceptible to photodynamic attack [38]. The inactivation of *Leishmania donovani* and *Trypanosoma cruzi* has been ably demonstrated by various groups in the area of blood photodecontamination (*q.v.*), using a range of photosensitisers [39-41]. However, it would be expected – as in the other areas discussed - that those with clinical pedigree would be employed in any therapeutic introduction. Methylene blue would thus be the front runner, along with crystal violet which was used conventionally until relatively recently for trypanosomal blood decontamination (i.e. without light activation) in South America [42,43].

Little is known concerning the effects of local photodynamic treatment on other protozoa, once again reflecting the paucity of research in this area. As with *L. donovani* and *T. cruzi*, and as discussed below, malarial parasites of the *Plasmodium* genus are very easily killed by photoactivated methylene blue. Consequently, it would be difficult to argue against similar predicted outcomes with other species of similar parasite. Indeed, it is notable that the photodynamic effect of methylene blue against *T. brucei* was first reported in 1938 [44].

Worm infection

As mentioned above, the concentration of photosensitiser required to kill a colonising worm/nematode would be relatively high due to the greater complexity of the organism compared to single-cell targets. However, individuals having undergone worm removal through the skin often suffer further from post-operative bacterial infection which, importantly, may delay the individual's return to work. Such infected sites are obvious targets for photodynamic disinfection as discussed elsewhere.

Blood product decontamination

The use of human blood, either directly in replacement or in fractionation to supply absent or under-provided protein factors is highly valuable in the saving or improvement of human life. However, its proper regional/national organisation is a considerable and expensive undertaking. Consequently, many of the areas subject to the diseases considered by the present discussion do not have access to the national blood service-type organisation enjoyed by most developed countries. Where this is possible, or is being developed, there is a part to be played by photosensitising dyes.

Human blood is a complex fluid, made up of both cellular and non-cellular fractions, the latter also being a complex mixture of proteins and other biological materials. Donated blood can be fractionated (red cells, platelets, plasma, clotting factors) or used whole, but there is obvious

potential for infection transmission (transfusion-transmission infection, TTI) if the original donation is pathogenically colonised, as explained above. As far as tropical disease is concerned, the major pathogens are bacterial, viral or protozoal in nature [45] (Table 2), although filarial transmission is also known [46], and this requires a very broad-spectrum approach, yet one which does not damage the various blood fractions contained.

Disease	Causative Organism	Presentation	Evidence
Bacterial			
Buruli ulcer	*Mycobacterium ulcerans*	Ulcer, skin & soft tissue Donated blood	*In vivo/in vitro vs. Mycobacterium* spp. [29]
Hansen's Disease (Leprosy)	*Mycobacterium leprae*	Infected digits/appendages	*In vivo/in vitro vs. Mycobacterium* spp. [27]
Trachoma	*Chlamydia trachomatis*	Eyes Donated blood	*In vitro vs. Chlamydia* spp. [34]
Yaws	*Treponema pallidum pertenue*	Ulcer, skin & soft tissue, bone Donated blood	*In vitro vs. Treponema* spp. [33]
Protozoal			
Chagas' disease	*Trypanosoma cruzi*	Ulcer, skin & soft tissue Donated blood	*In vitro vs. Trypanosoma* spp. [68-70]
Human African Trypanosomiasis	*Trypanosoma* spp.	Donated blood	*In vitro vs. Trypanosoma* spp. [44]
Cutaneous leishmaniasis	*Leishmania* spp.	Ulcer, skin & soft tissue Donated blood	*In vitro vs. Leishmania* spp. [67]
Viral			
Chikungunya	Alphavirus (enveloped)	Donated blood	*In vitro vs*. virus [73]
Dengue	Flavivirus (enveloped)	Donated blood	*In vitro vs*. virus [73]
Rabies	Lyssavirus (enveloped)	Donated blood	*In vitro vs*. virus [75]

Table 2. Suitable presentations for photodynamic attack

A further complication pertains to the exact situation of the infecting organism in cellular fractions, since this might be intracellular, cell-associated (externally) or diffuse in the suspending medium (plasma). Logically, the ease of targeting increases in the same order. For example, the use of methylene blue and red light to inactivate dengue virus would be relatively straightforward in plasma, but is much less effective in red cell concentrates due to the fact that the dye does not readily cross into the cell interior [47]. Indeed, methylene blue causes red cell membrane damage on

illumination [48]. Alternative approaches – e.g. ultraviolet illumination, ultrafiltration or solvent-detergent treatment – all have drawbacks, usually relating to specificity and collateral damage [49].

The use of dyes for the disinfection of donated blood was suggested in 1955 [50], again, methylene blue being the dye in question due to its considerable prior use in humans. This did not become a reality until the 1990s, but since then many millions of plasma units have been treated in Europe [51].

As noted above, the HIV-AIDS pandemic, commencing in the late 1970s, led to a re-examination of blood donation processes to ensure that viral contamination could not occur. In turn, the ensuing protocols introduced gave protection against many other pathogens. The very broad-spectrum range provided by the photodynamic approach obviously covers most current pathogenic bacteria, fungi, viruses and protozoa, including emerging pathogens of these types. However, this is most effective in the treatment of non-cellular blood products (Figure 5). The Zika virus is of considerable concern currently, mainly due to resulting birth defects, and this can be transmitted via donated blood [52]. However, being an enveloped virus, of the Flavivirus family (like the yellow fever virus, for example) Zika should be susceptible to the photodynamic approach, using methylene blue or riboflavin - the first report of the photodynamic inactivation of yellow fever virus (with methylene blue) occurred as far back as 1934 [53]. The range of blood component-susceptible pathogens relevant to the current argument is shown in Table 3, again demonstrating both the dearth of research in this area and the potential for new protocol development.

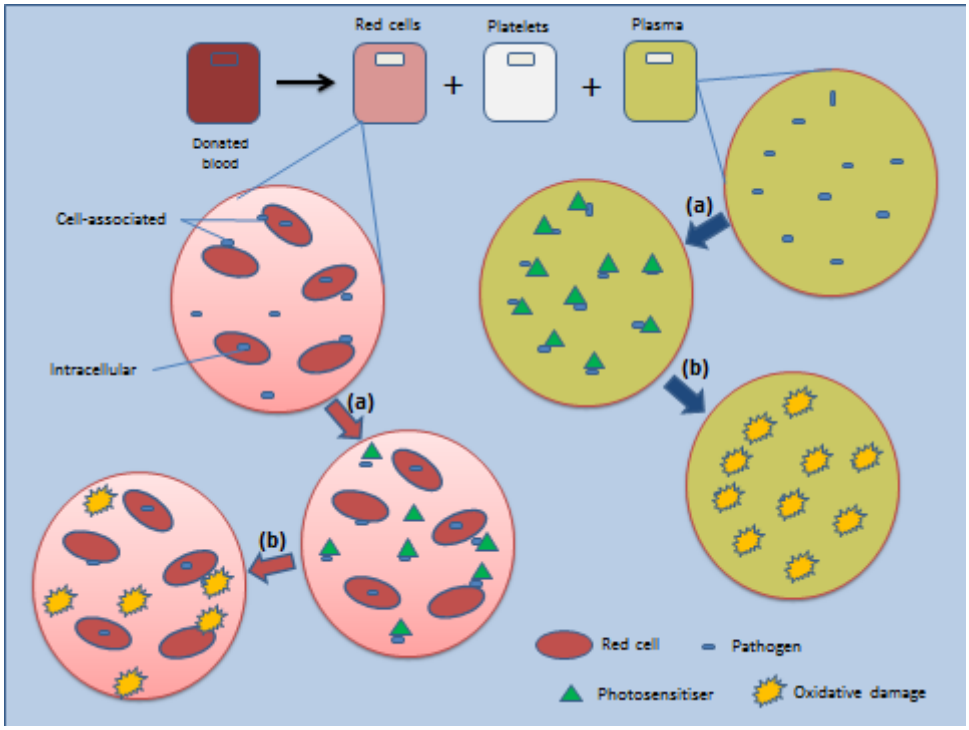


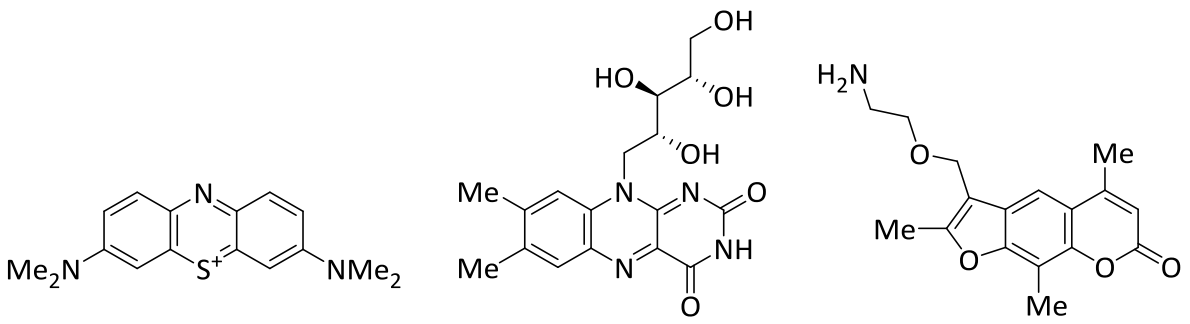
Figure 5. Pathogen photoinactivation process for red cell and plasma fractions. In each case, the fraction is incubated with a photosensitiser (e.g. methylene blue or riboflavin, step **(a)**) and then illuminated **(b)**. Clearly the process is more straightforward in the absence of cellular material.

Pathogen	Photosensitiser	Fraction
Leishmania donovani	Riboflavin	Plasma / Platelets [67]
Trypanosoma cruzi	Methylene blue	Plasma [68]
	Riboflavin	Plasma / Platelets [69]
	Crystal violet	Whole blood [70]
	Phthalocyanines	Red cells [71]
Plasmodium falciparum	Silicon phthalocyanine (Pc4)	Red cells [72]
Chikungunya	Methylene blue	Plasma [73]
	Riboflavin	Plasma / Platelets [74]
Dengue	Methylene blue	Plasma [73]
Rabies virus	Methylene blue, proflavine	Plasma [75]

Table 3. Reported blood component photodisinfection capabilities *versus* causes of various tropical diseases

As can be seen, there are currently few dyes suitable for use in blood product decontamination, however wide the range for methylene blue, and this is certainly an area which requires proper examination via organised research. Riboflavin (vitamin B2) and the psoralen (furanocoumarin) derivative amotosalen (Table 3, Figure 6) should not be counted as conventional dyes, although they do act as photosensitisers. The scientific argument for riboflavin use lies in its lack of toxicity, being an essential human vitamin [54]. Conversely, amotosalen is an entirely synthetic agent which has been shown to have very low toxicity in mammals and works via photochemically crosslinking pathogenic DNA or RNA [55]. Similarly to the methylene blue process, further insurance against toxicity is provided by the removal of the psoralen by a post-illumination adsorption step (i.e. before transfusion).

The exploitation and development of these few photosensitised/photochemical processes has been carried out commercially by MacoPharma (methylene blue / Theraflex system), Terumo BCT (riboflavin / Mirasol) and the Cerus Corporation (amotosalen / Intercept) [56-58].



Methylene blue Riboflavin Amotosalen

Figure 6. Photosensitisers in current use for pathogen inactivation.

Water purification

The use of the photodynamic approach to pathogen inactivation is most effective with simple cell types, rather than complex, or multicellular organisms. Logically, the greater the target complexity, the more damaging events are required for its inactivation. Consequently, for example, simple prokaryotic bacteria are far more susceptible to the approach than are healthy eukaryotic cells, such as those found in the human or animal host. Many tropical pathogens have a relatively simple, aqueous stage which, in some cases leads to infections due to far more complex organisms, such as the larval flatworms associated with schistosomiasis [59]. A similar situation often pertains to the arthropod vectors of disease, such as mosquito eggs and larvae. These more rudimentary targets can be combatted in their aqueous environment using a photodynamic approach which can be seen as a modification of the blood product decontamination method discussed in the previous section.

Clearly, the treatment of a large volume of colonised water offers a different prospect to that of a plasma blood bag. However, at both extremes, the product must be 'clean' enough for human use in the absence both of pathogenic contamination and of the treating photosensitiser to prevent any post-treatment toxicity. While this is achieved in blood product disinfection by small adsorption tubes, such an approach for large-scale work would be unwieldy and expensive. In addition, it would be a far 'greener' approach to be able to re-use the photosensitiser for subsequent water treatments. This might be achieved by attaching the photosensitiser to a solid matrix, either fixed within the decontamination chamber or, perhaps in granular form, moved throughout the medium during the agitation associated with the illumination process. Photosensitiser matrices would be retained via filtration of the treated water. Such a scenario is shown in Figure 7.

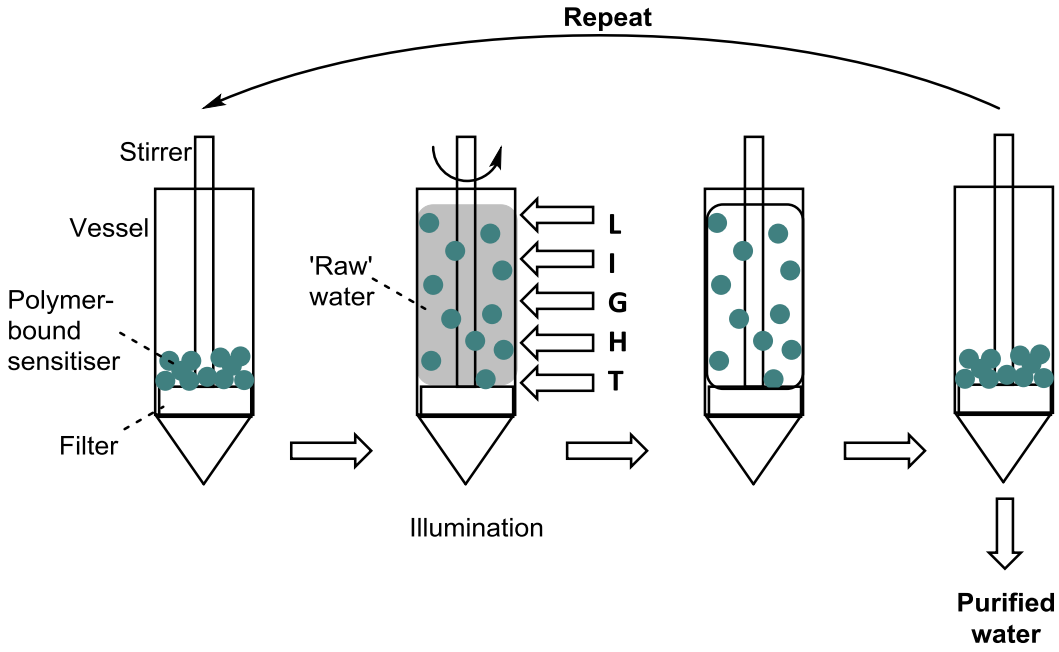


Figure 7. Potential water photodecontamination process.

The provision of potable water is essential to health and liberal estimates put the annual global mortality associated with the lack of this resource at 502,000 diarrhoeal deaths [60]. Given a robust photosensitising system and, in many cases, a very reliable and free (solar) light source, the photodynamic approach to water purification should be a relatively straightforward proposition, particularly in the case of associated enteric bacterial pathogens such as *Escherichia coli*, *Shigella* and *Vibrio* spp., given their established susceptibilities [61-63]. Industrial water treatment has been carried out using the inorganic photosensitising pigment anatase (TiO_2) [64], but organic photosensitisers offer a wider range of visible absorption – anatase uses ultraviolet. In addition, given that the photosensitisers would be retained during the water processing, toxicity requirements would presumably be less stringent.

Do it yourself

Much of the preceding commentary concerning the use of photoactivable agents to combat infectious disease pertains to photosensitisers and light sources which would require purchase by healthcare agencies in the afflicted regions. In this respect, the same fiscal problems would arise which currently inhibit the provision of conventional drugs, although to a lesser degree, given the relatively inexpensive nature of established photosensitisers such as methylene blue and crystal violet. In other words, developing nations would remain dependent on multinational corporations from the industrialised nations for their healthcare.

The synthesis of the dyes, materials or equipment proposed would not be of great cost to the end-user, if it were possible for these to be produced within the regions requiring them, possibly with the advice or collaboration of overseas academics or industrialists with prior experience in the

preparation and application of photoantimicrobials. Such local development/production would also allow the tailoring of therapy to suit the local environmental conditions.

A regular, metred amount of light is preferable for safe therapy, e.g. from a laser or light-emitting diode (LED) array. However, as noted above, since the diseases under discussion are mainly suffered in equatorial regions, there is also a plentiful and reliable supply of solar radiation. Clearly, this might be used where metred light is not available to promote the photodynamic treatment of local infection, but care must be taken both to match the radiation to the photosensitiser and to avoid side effects. The former criterion is relatively straightforward, since the photosensitisers available for clinical use have well-known absorption profiles. For example, methylene blue, toluidine blue and crystal violet all possess intense, long-wavelength absorption bands (600-670 nm region) which overlap with the long-wavelength region of the solar spectrum. Side effects might arise, for instance in the treatment of localised cutaneous infection, from the exposure of healthy tissue to the photosensitiser and sunlight, but this can be avoided by covering tissue in the peripheral regions (e.g. with black surgical tape), so that the infected region is exposed precisely. In addition, the amount of light incident on the target area can be measured or modelled and solar exposure times recommended.

Furthermore, many plants are known to contain photosensitising chemicals [65]. It is possible to decoct mixtures of e.g. psoralens and coumarins from various plant parts and to apply these as photodynamic, though less regulated, preparations to infected sites. This approach has been used since ancient times – e.g. in Egyptian and Ayurvedic medicine – for the treatment of skin disease [66]. However, since such compounds are not strictly dyes, they fall outwith the current area of review.

Conclusions

Tropical medicine is under-funded in the 21st Century. Pressures usually generated in the more fortunate parts of the global economy mean that health spending *per capita* in many countries blighted by the diseases covered here is a small fraction of that available in developed nations. Consequently, effective alternative approaches to the treatment of neglected illnesses such as cutaneous leishmaniasis or Chagas' disease offer considerable scope for the improvement of health, particularly given the decreased costs involved. There is much to be discovered concerning the use and general applicability of photosensitisers in tropical medicine and there are clearly a number of diseases which are suited to being treated by this approach.

Given the lack of properly organised research and development possible in most countries of the Developing World, it is incumbent on richer economies – preferably in firm partnership with these countries – to deliver suitable therapeutics. Such action would be particularly fitting, given that it was the pursuit of the chemotherapy of tropical disease that allowed the discoveries which led to the formation of the pharmaceutical industry.

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