'Safe' photoantimicrobials for skin and soft-tissue infections

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ARTICLE INFO

Article history:
Received 7 December 2009
Accepted 3 March 2010

Keywords:
Drug resistance
Erythrosine
Indocyanine green
Methylene blue
Non-toxic dyes
Phloxine B
Photoantimicrobials

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ABSTRACT

In light of increasing bacterial drug resistance, novel agents and/or modes of bactericidal action are required. In particular, agents that can act at multiple sites within the bacterial cell appear to offer less potential for the development of resistance. This effect may be achieved using combinations of established drugs. Similar effects have been reported as a result of photodynamic antimicrobial action alone. The photoactivation requirement suggests the utility of such agents in light-accessible presentations such as skin and soft-tissue infections. This approach also offers considerable potential in decreasing conventional antibacterial use and allowing the conservation of important life-saving systemic agents.
1. Introduction

Dyes that were shown to demonstrate bacteria in the presence of mammalian cells were the progenitors of modern chemotherapeutic agents. Work by Gram, Koch, Ehrlich and others provided a sound basis for the idea of selective toxicity, and several biological stains were introduced as antimicrobial agents in the early part of the last century, namely the acridines proflavine and acriflavine against bacterial infection and the triphenylmethanes crystal violet and brilliant green against both bacterial and fungal infection [1]. Whilst the use of such agents caused tissue staining, many lives were saved, for example during the First World War where ‘flavine therapy’ was employed in battlefield injuries [2]. Agents such as the acridine dye acriflavine were also tested as systemic (oral) preparations for gonorrhoea, the highly hydrophilic nature of the dye ensuring rapid elimination [3].

Whilst the use of dyes in antimicrobial chemotherapy was naturally cut short by the emergence of penicillin and the modern antibacterials, the use of colour in tissue differentiation has never significantly decreased. Intraoperative use of stains such as methylene blue normally employs aqueous solutions of the dye at typical concentrations of 1% w/v [4]. This concentration of methylene blue (intravenous) is also indicated for the treatment of methaemoglobinemia, both uses thus demonstrating the low toxicity associated with the phenothiazine derivative. From an antimicrobial point of view, methylene blue has also been administered in the treatment of juvenile falciparum malaria in a significant trial in Burkina Faso [5], whilst the related
dye gentian violet has been used in the treatment of clinical meticillin-resistant *Staphylococcus aureus* (MRSA) infection in Japan [6].

Use of dyes in a clinical setting is thus possible, but there remains the perceived problem of colouration where this is not the intended effect. However, there must be a cost–benefit balance made if other treatments are unsuccessful. The use of gentian violet against MRSA provides a good, if relatively rare, example of dye use, yet pre-operative iodine use remains ubiquitous. Given the continuing rise in microbial drug resistance, it seems logical to consider active dyes as local therapeutics where possible and also to use them in order to conserve more valuable systemic agents.

In this area, an enormous potential impact should be made by exploiting the photodynamic effect associated with a range of dye types, some examples of which are already in clinical use in humans for conventional purposes. Photodynamic agents, or photosensitisers, use light energy to promote the formation of reactive oxygen species (ROS) such as singlet oxygen, superoxide, hydroxyl radicals etc. in situ (Fig. 1). Such species represent a considerable toxic threat to simple microbial cells.

2. **Photoantimicrobials**

As noted above, there are various dyes that pre-date conventional drugs as antimicrobials. Since the first literature report of photoantimicrobial action was published in 1900, this too has a longer history [7]. However, for the reasons mentioned previously, photoantimicrobial agents, which were also dyes, were
left behind in terms of their clinical use during what is often called the ‘golden age of antibiotics’. Some comparison of the development of photoantimicrobials with that of conventional agents is possible from Fig. 2.

Plainly, the level of investment made by the pharmaceutical industry in developing antimicrobial chemotherapeutics is enormous. Consequently, the sites and modes of action of modern agents are understood in exquisite detail; indeed, such is the level of understanding that for several decades drug molecules have been specifically designed to be target-specific. As noted in the introduction, this degree of sophistication, while allowing targeting at the molecular level, also exerts selective pressure and promotes microbial resistance development.

Photoantimicrobial agents are not single-target specific. Production of highly reactive singlet oxygen in situ means that a range of biomolecular targets is available in the immediate environment of the photosensitiser (structural proteins, enzymes, nucleic acids, unsaturated lipids etc.) via the diffusion of ROS. Consequently, activity against conventional drug-susceptible microbes is also observed for strains that are conventional-resistant [8,9]. Multiple sites of therapeutic action can be achieved by employing combinations of conventional drugs, as already mentioned, but again this is based on defined, rather than variable, sites of action. In addition, the practicalities of combining different drugs may be self-defeating (drug–drug interactions, contraindication, formulation etc. and, of course, cost).
As an example, the standard photosensitiser methylene blue has been shown to cause photodamage in the Gram-negative organism *Escherichia coli* at the level of the outer membrane [10], cell wall [11], ribosome [12] and nucleic acid [13]. To achieve such varied targeting with conventional agents would require the combination of, for example, a peptide antibiotic, a β-lactam agent, a tetracycline and a fluoroquinolone.

Since there is, by definition, a light requirement for the application of photoantimicrobial agents, there is a concomitant limit in terms of potential sites where the approach may be exploited, but this certainly covers skin and soft-tissue infections (SSTIs). Efficient light sources need no longer be of the expensive laser type; there are many highly suitable (and inexpensive) examples based on light-emitting diodes (LEDs). However, there is currently no mechanism that would allow the systemic use of photoantimicrobials, although where there is a localised SSTI or undesirable colonisation they should prove useful, particularly from the angle of conventional antimicrobial conservation.

### 2.1. Permitted agents

The principal dye/photosensitiser in terms of human use is methylene blue, this compound having been employed in various applications, both indicative and therapeutic, since the late 19th century [14]. Other photosensitisers with everyday application in humans include the triphenylmethane dye gentian (crystal) violet and the food colourings erythrosine and phloxine B. Similarly, indocyanine green has various applications in clinical blood flow measurement.
[15] (see Table 1). Any or all of these examples could be utilised with relatively little difficulty from a regulatory point of view, since each is already in use in humans, and very seldom with any control of incident light. There are many newer, improved photosensitisers available as a result of two decades or more of concentrated research [16], but these will require considerable investment in time and money in order to reach the clinic. Introduction of these improved agents will depend very much on acceptance of the ‘standard’ photosensitisers listed above.

The related anticancer approach employing photosensitisers is known as photodynamic therapy (PDT). The agents used here are porphyrin-based—haematoporphyrin derivatives, chlorins or porphyrin precursors [17]. Whilst these are, happily, being increasingly licensed in specified cancers, their selectivity for microbial cells is limited and, consequently, they are not covered in this discussion.

Among the ‘standard photosensitisers’, the use of both methylene blue and crystal violet has been recounted above. Both erythrosine and phloxine B are globally accepted food colourings, whilst the former is also the staining constituent in dental plaque disclosing tablets. Conversely, indocyanine green, as well as being employed in blood volume/blood flow measurement, is a near-infrared absorber and has been shown to act photothermally in tissue soldering [18] (Table 1). Product familiarity via clinicians’ first-hand experience of these materials can only help to promote their use as photosensitisers.
2.2. Photoantimicrobial activities

As indicated in Table 2, both of the cationic (positively-charged) photosensitisers, methylene blue and crystal violet (Fig. 3), are established broad-spectrum antimicrobials, i.e. active against all four major classes and subdivisions of microbial pathogens. The remaining agents in current human use are all associated with an overall anionic charge (Fig. 3) and this is established as a predisposing factor for lack of photobactericidal efficacy against Gram-negative organisms [19].

It is evident from Table 2 that there is a considerable knowledge gap concerning erythrosine, phloxine B and indocyanine green with regard to active range. However, it has been reported that negatively-charged photosensitisers such as the related cyanine derivative merocyanine 540 are active against some viruses [20]. Similarly, since the two xanthene derivatives erythrosine and phloxine B have been reported to be effective against Gram-positive oral pathogens such as Streptococcus mutans [21], and indocyanine green is reportedly highly active against S. aureus and Streptococcus pyogenes [22], this suggests their use against other Gram-positive organisms. Erythrosine has also been reported to be toxic towards the yeast Saccharomyces cerevisiae [23]. Brief data for the compounds regarding both mammalian use/dose, in vitro photoantimicrobial activities and light dose are given in Table 1. Reports of the use of photosensitisers in either human or animal infections are very scarce, although methylene blue has been used successfully (topical, 2% w/v, red light) in the treatment of onychomycosis [24] and in a murine model of oral candidiasis (local, 0.05% w/v, red light) [25].
Data from many more trials currently remain subject to industrial confidentiality.

3. Conclusion

Photosensitisers such as methylene blue and crystal violet have been shown to inactivate all of the bacterial species mentioned in the preceding paragraphs. Given the current state of knowledge regarding structure–activity relationships in photosensitisers, it would not be ridiculous to predict highly effective action against significant Gram-positive organisms for the remaining ‘human-safe’ photosensitisers covered here.

According to recent data, SSTIs constitute one-fifth of hospital-associated infections in England [26]. Given that many SSTIs offer relatively facile topical access, the use of photosensitisers here, in combination with superficial illumination, surely offers considerable potential for infection control. Such an approach has advantages both from the point of view of the lack of likely bacterial resistance and also in the resulting decrease in the use of conventional antimicrobial agents, thus allowing their conservation for more complicated or life-threatening systemic disease.

Funding
None.

Competing interests
None declared.
Ethical approval

Not required.
References


Fig. 1. Photoantimicrobial mechanisms.

Fig. 2. Timelines for conventional drug and photoantimicrobial discovery. MB, methylene blue; PF, proflavine; NR, neutral red; HpDA, haematoporphyrin–arginine conjugate; MRSA, meticillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant enterococci; Ce6PL, chlorin e6–polylysine conjugate.

Fig. 3. Proposed ‘first-generation’ photoantimicrobials.
### Table 1

Human usage/dosage of proposed photosensitisers and in vitro photoantimicrobial activities [as minimum bactericidal concentration (MBC)]

<table>
<thead>
<tr>
<th>Photosensitiser</th>
<th>Human use</th>
<th>Concentration (μM)</th>
<th>Reference</th>
<th>Organism</th>
<th>MBC (μM)</th>
<th>Light</th>
<th>Light dose (J/cm²)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylene blue</td>
<td>Methaemoglobinemia</td>
<td>30 000</td>
<td>[36]</td>
<td>MRSA</td>
<td>20</td>
<td>White</td>
<td>6</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>Bladder tumour stain</td>
<td>30 000</td>
<td>[4]</td>
<td><em>Escherichia coli</em></td>
<td>100</td>
<td>White</td>
<td>6</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td>Sentinel node tracing</td>
<td>30 000</td>
<td>[37]</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>25</td>
<td>White</td>
<td>6</td>
<td>[38]</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>Topical antisepsis (MRSA)</td>
<td>2500</td>
<td>[6]</td>
<td><em>Streptococcus sanguinis</em></td>
<td>250</td>
<td>HeNe laser</td>
<td>1</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>Candida albicans</em></td>
<td>250</td>
<td>HeNe laser</td>
<td>1</td>
<td>[40]</td>
</tr>
<tr>
<td>Tissue soldering</td>
<td>3000</td>
<td>[18]</td>
<td>Streptococcus pyogenes</td>
<td>32</td>
<td>810 nm laser</td>
<td>411</td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------</td>
<td>------</td>
<td>------------------------</td>
<td>----</td>
<td>--------------</td>
<td>-----</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>Erythrosine</td>
<td>Disclosure tablets</td>
<td>24 000</td>
<td>[21]</td>
<td>Streptococcus mutans</td>
<td>22</td>
<td>White</td>
<td>20</td>
<td>[21]</td>
</tr>
<tr>
<td>Phloxine B</td>
<td>Food colouring</td>
<td>Variable</td>
<td>–</td>
<td>MRSA</td>
<td>30</td>
<td>White</td>
<td>a</td>
<td>[41]</td>
</tr>
</tbody>
</table>

MRSA, meticillin-resistant *Staphylococcus aureus*.

a Standard fluorescent room lighting.
**Table 2**

Photoantimicrobial activities of photosensitising dyes currently in human use

<table>
<thead>
<tr>
<th>Photosensitiser</th>
<th>Bacteria</th>
<th>Fungi</th>
<th>Viruses</th>
<th>Protozoa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram-positive</td>
<td>Gram-negative</td>
<td>Yeast</td>
<td>Mould</td>
</tr>
<tr>
<td>Methylene blue</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Crystal violet</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+ N/R</td>
</tr>
<tr>
<td>Erythrosine</td>
<td>+</td>
<td>–</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Phloxine B</td>
<td>+</td>
<td>–</td>
<td>N/R</td>
<td>N/R</td>
</tr>
<tr>
<td>Indocyanine green</td>
<td>+</td>
<td>–</td>
<td>N/R</td>
<td>N/R</td>
</tr>
</tbody>
</table>

+, reported activity; –, reported inactivity; N/R, not reported.
Edited Figure 3

Methylene blue

Crystal violet

Erythrosine

Phloxine B

Indocyanine green