PERSPECTIVE

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Photosensitized inactivation of microorganisms

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Despite major advances in medicine in the last 100 years, microbiologically-based diseases continue to present enormous global health problems. New approaches that are effective, affordable and widely applicable and that are not susceptible to resistance are urgently needed. The photodynamic approach is known to meet at least some of these criteria and, with the creation and testing of new photosensitisers, may develop to meet all of them. The approach, involving the combination of light and a photosensitising drug, is currently being applied to the treatment of diseases caused by bacteria, yeasts, viruses and parasites, as well as to sterilisation of blood and other products.

Introduction

The fact that many human and animal diseases can be caused by micro-organisms has been recognised for many centuries. In the last 150 years, there has been a huge increase in knowledge of the natural history of the micro-organisms themselves and how they are implicated in the transmission of disease. Treatments have been developed whereby micro-organisms are selectively destroyed, resulting in the cure and sometimes complete elimination of previously incurable diseases.[1] The development and widespread use of antibiotics to treat bacterial infections represents one of the most revolutionary advances ever made in scientific medicine. Understanding of the transmission of malaria and development of antimalarial therapies has produced major benefits. The development of good water supplies and hygiene-based procedures for a whole range of human activities (at least in the developed world) has reduced the likelihood of transmission of microbiological disease.[2]

Against this background, it might have been expected that microbiologically-based disease at the beginning of the twenty first century would have been reduced to a level that no longer had a serious impact on human health. In reality, this is far from the case. Resistance has developed to antibiotics which were previously highly effective, medical science has failed to find a comprehensive therapy to combat viral pathogens and new agents such as prions have emerged. Poverty in the third world has prevented the adoption of good practice in combating disease. This, coupled with frequent travel, means that transmission of micro-organisms has become a global phenomenon.

There is now an urgent need for the development of novel, convenient and inexpensive measures for combating microbial disease. Photodynamic technology may provide one approach to meeting this need, both in terms of therapy and in terms of sterilisation.[3,4]

Photodynamics is a platform technology which uses a combination of a photosensitiser, light and molecular oxygen to achieve selective destruction of a biological target.[5] The principle behind the approach is illustrated in the following scheme:

$$\text{Sensitiser} + \text{light} \longrightarrow \text{activated sensitiser}$$
$$\text{Activated sensitiser} + \text{oxygen} \longrightarrow \text{sensitiser} + \text{activated (singlet) oxygen}$$
$$\text{Activated oxygen} + \text{target} \longrightarrow \text{oxidised (damaged) target}$$

Energy from light is absorbed by the photosensitiser and then passed on to molecular oxygen with the formation of the very reactive singlet oxygen. It is singlet oxygen which is the agent which causes potentially lethal damage to the target. During

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this process, the photosensitiser is regenerated so that it acts as a kind of catalyst and many molecules of singlet oxygen can be formed from a single molecule of photosensitiser, so long as light and molecular oxygen are present.

Photodynamic technology has now been extensively developed for therapeutic purposes to selectively destroy mammalian lesions such as cancers and five photosensitising drugs have now been licensed for general use.[6] Although there has yet been little application of photodynamic technology for the selective destruction of micro-organisms, it has recently become clear that this field has a high potential, both for therapy and for non-therapeutic purposes such as sterilisation.

Bacterial photoinactivation

Bacteria fall into two main classes depending upon their response to the Gram stain, which reflects differences in their morphology as illustrated in Fig. 1. Gram negative and positive bacteria differ in the composition of their outer surface, and respond differently to antimicrobial agents.[4,7] Gram positive bacteria can easily take up molecules such as photosensitisers and can therefore be readily photoinactivated by most photosensitisers used for conventional PDT. This is not the case for Gram negative bacteria however, which are relatively impermeable to neutral or anionic drugs due to their highly negatively charged surface.[8] Since almost all photosensitisers which have previously been developed for PDT come into this category, this explains why these photosensitisers are not effective alone against Gram negative bacteria. However, it has been shown that many of these photosensitisers can become effective against Gram negative bacteria when they are co-administered with a cationic agent such as polymixin.[9] The latter agent is able to disrupt the cell wall of the bacterium sufficiently to permit access of the photosensitiser which can then cause lethal damage to the cell when it is exposed to light.

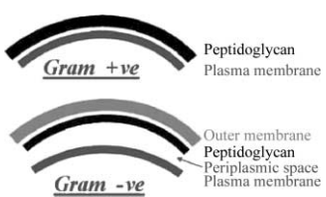

Fig. 1 A diagram showing the differences in cellular structure between Gram positive and Gram negative organisms.

Clearly it would be desirable to have an effective photosensitiser for Gram negative bacteria without the need for co-administration of a disrupting agent. This was achieved in work carried out simultaneously in Padova with cationic porphyrins and in Leeds with cationic phthalocyanines.[10,11] Both of these classes of photosensitiser were active against Gram negative as well as Gram positive bacteria, probably because the cationic sensitisers had a dual action, first in disrupting the bacterial cell wall and then in subsequently photosensitising the cells. Recent studies[12] showed that non-cationic photosensitisers, such as chlorins, can efficiently promote the photoinactivation of Gram-negative bacteria, provided they are covalently bound to a poly-lysine oligomer, which is positively charged at physiological pH values.

The above outlined picture does not apply to mollicutes, a class of bacteria, which are also named mycoplasmas. Mollicutes are genetically deficient of cell wall structures. As a consequence, the cytoplasmic membrane is readily accessible to photosensitising agents and these cells are susceptible to photoinactivation independently of the charge of the photosensitiser molecule; rather, the degree of photosensitivity of mollicutes is correlated with their content in cholesterol, which they can readily accumulate from the medium. Thus, cholesterol-rich *Mycoplasma hominis* is significantly less photosensitive than cholesterol-deprived *Acholeplasma laidlawii*.[13]

Photoinactivation of viruses

Viruses represent a completely different type of target for PDT compared with bacteria, but the principle governing effective treatment remains the same *i.e.* the viruses must be destroyed without causing unacceptable damage to the host cells. Although the photoinactivation approach has been known for many years, it has so far found only very limited clinical application in terms of therapy. Since PDT is a local technique, it would seem that the best approach would be the targeting of localised viral disease and particularly those lesions which may progress to malignancy. Again, the design and mode of action of the sensitiser is critical in this field and this must be linked to the structure and function of the particular viral target.

In the decontamination of blood products, antiviral photodynamic technology is rather further advanced with at least two products being used commercially. This is also a very active area of research with new photosensitisers being developed and assessed. The current and future use of the photodynamic approach in the treatment of viral disease and in the decontamination of blood and other biological products is discussed in detail in the following article by Wainwright.

Photoinactivation of other micro-organisms

Microbial cells exhibit a large variety of size, subcellular architecture, biochemical composition and susceptibility to externally added chemical agents. In spite of such a great diversity, the pathways leading to the eventual photosensitised inactivation of various types of microbial cells are very similar with those described for the photoinactivation of bacteria, especially in those most frequent cases where the outer wall does not act as a tight permeation barrier as it is typical of Gram-negative bacteria.

Thus, yeasts and fungi, such as *Candida albicans* and *Saccharomyces cerevisiae*, are eukaryotic cells surrounded by a relatively loose external membrane; therefore, even anionic photosensitisers, such as haematoporphyrin, are effective photocidal agents.[14] However, the efficiency of the photosensitised process is markedly more pronounced by the conjugation of the porphyrin molecule with glycosyl moieties which enhance their penetration into inner cellular districts.[15]

Lastly, recent findings[16] appear to indicate that photodynamic techniques can be usefully applied for the inactivation of parasitic pathogens. As it is well known, several phyla of protozoa have developed a parasitic lifestyle, and some of them are quite dangerous and deadly pathogens for humans.[17] Some human parasites, such as *Giardia intestinalis*, *Naegleira fowleri*, *etc.* present at least one stage in their life cycle outside the body of the host and can be transmitted between different individuals by food and water contamination. Moreover, parasitic infections can be transmitted through blood transfusion, such as those caused by *Plasmodium* (malaria), *Babesia* (babesiosi) and *Trypanosoma* (*e.g.*, sleeping sickness). Several physical and chemical approaches are used to inactivate parasites; however, the level of disinfections are often limited, and some conventional treatments may also generate harmful effects on the environment.[18] On the other hand, novel perspectives could be opened by the utilization of photodynamic processes promoted by cationic porphyrins and phthalocyanines: such photosensitisers are phototoxic on parasitic and free living protozoa in both the cystic and vegetative stage of their development,[16,19] and have been shown to be active against blood-born pathogens involved in tropical diseases,[19] as well as against a pathogenic amoeba (*Acanthameba palestinensis*) which is the causative agent of granulomatous encephalitis and some chronic eye infections.[20] Such a broad spectrum of antimicro-

bial and antiparasitic activity makes photodynamic techniques quite promising also for the disinfection of microbiologically contaminated water.[21]

Future potential of photodynamic technology in the anti-microbial field

The large body of laboratory evidence which is now accumulating suggests that photodynamic technology may potentially play a major role in antimicrobial therapy as well as in non-therapeutic applications. With the exception of small and largely anecdotal studies with methylene blue and a very small number of other photosensitisers, the field remains wide open for further development. In the therapeutic arena, there is a strong potential for the use of PDT in the treatment of a range of dermatological conditions such as infected ulcers, infected burn wounds and skin disease involving microorganisms. This will require topical applications of photosensitisers which will be selective for the microorganism, without causing unacceptable damage to the host tissue. Applications in the dental field have already been investigated and are likely to become established as improved sensitisers are developed. There are many other therapeutic applications which can be imagined, some of which are considered in the following papers.

Sterilisation of blood products and other organ transplant tissue is another area which lends itself to the photodynamic approach. Again, the key factor is the ability to destroy a wide range of pathogens, without significant damage to the tissue to be transplanted. More general applications in the sterilisation field are also likely, including sterilisation of surfaces and sterilisation of air and water.

In conclusion, this is a field which has a huge potential and which has been made much more accessible by recent progress in photodynamic technology. The following papers illustrate some of the many applications which are now ready for further development and we may expect to see many more in the future.

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