Chemistry, Light and Oxygen to fight antimicrobial infections

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Bad bugs no drugs

There is a preconceived concept that associates bacteria with pathogenesis and, in the end, with deleterious effects. It is estimated that there are approx. 1,400 known species of human pathogens, but they account for much less than 1% of the total number of microbial species on the planet. Despite this low number, pathogenic microorganisms have become a universal threat. The extensive and inappropriate use of antibiotics has gradually led to a worrying resurgence of morbidity and mortality from new and old infectious diseases.[1] Today infectious disease is the second most important killer in the world: worldwide, 17 million people die each year from bacterial infections. The emergence of the so called *superbugs* or ESKAPE pathogens cause serious and life-threatening infections: they are extremely difficult and in many cases impossible to effectively treat.[2] Thus, alternatives to antibiotics are badly needed.

Photodynamic Therapy or the "one for all, all for one"

"I don't understand it Dr. Von Tapeiner, The paramecia were all wiggling just fine a minute ago, but now these over by the window seem to be dead."

-Oscar Raab (1900)

Although there are several accounts of photosensitization reactions in the writings of the Egyptians, Indians and Chinese dating to at least 30 centuries ago, the origin of photosensitization as a science is usually attributed to the work of Oscar Raab, a medical student of Hermann von Tappeiner, who was involved in a study of the toxicity of acridine dye on paramecia. In the winter of 1897-98, Raab found that the apparent toxicity that he measured was quite variable. Specifically, it seemed to depend on the time of day when he performed his experiments! He hypothesized that one of the variables in his work was the amount of light, and he subsequently demonstrated that, indeed, paramecia were inactivated more effectively if the acridine solutions were kept near a bright window, than if they were not exposed to light. The surprising result was published in 1900 and stimulated further activity in the field.[3] It was the first report regarding what they called *photodynamic action*. Since then, a lot has been learnt about this effect and the development of its use in therapies is a fact.

The paradigm of photodynamic therapy (PDT) comprises the action of three players: a light-active molecule called photosensitiser (PS), a light source of appropriate wavelength and oxygen. None of them are toxic *per se*; however, their combination renders the production of reactive oxygen species (ROS) responsible for exerting damage to malignant cells:[4] *one for all, all for one*! Among these ROS, singlet oxygen ($^1O_2$), the lowest electronically-excited state of molecular oxygen, is endowed with rather unique properties especially relevant for application in biological systems: it is small and therefore capable of diffusing with relative ease; it is non-charged, which allows it to cross membranes; it is fairly reactive (almost every cell component is a potential target) and there are no known antioxidant enzymes for removing it. All these attributes have proven useful in different fields, especially in the battle against cancer or antimicrobial diseases.[5]

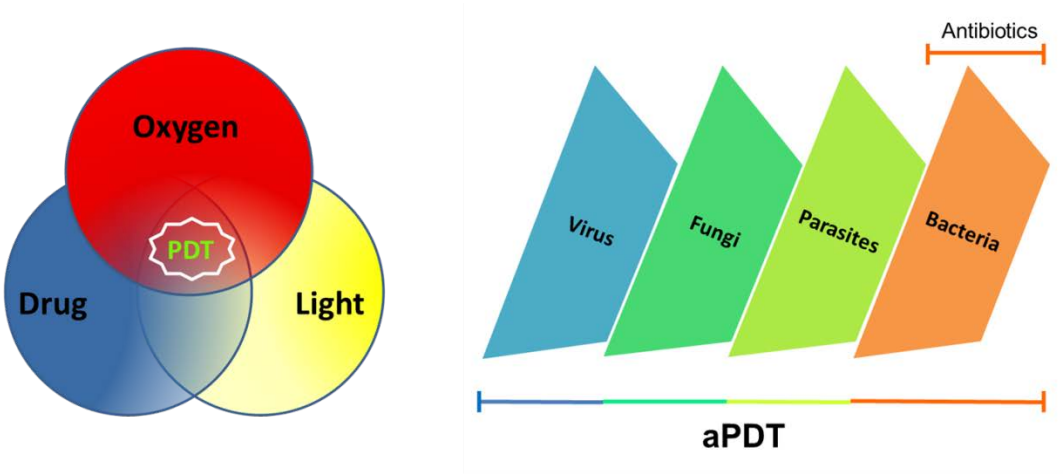

Fig. 1 The paradigm of PDT and the broad-spectrum of action of aPDT.

PDT is highly selective as cell damage is spatially limited to regions where light is applied. Moreover, when PDT is applied to treat antimicrobial infections (hereafter aPDT) it presents broad-spectrum activity (it is active against virus, fungi, protozoa and bacteria, including antibiotic-resistant strains) and a lack of development of resistance mechanisms due to its multi-target mode of action. These features make aPDT a potential candidate over traditional antimicrobials.[6]

Singlet oxygen and fluorescent proteins: Trojan horses killing bacteria from inside

One of the problems of classical PDT is achieving proper spatio-temporal control of $^1O_2$ generation. Synthesis of the PS, its delivery and internalization are key events in dictating success in a photodynamic treatment. How nice it would be if we didn't need to synthesize the PS and, instead, we could let the cells do the work for us. Whenever I come to think of it, it reminds me of a Trojan Horse. Giving bacteria instructions to make the PS -a tainted present-, letting them grow and finally shedding light to exert localized damage. Imagination or reality?

The 2008 Chemistry Nobel Prize recognized the discovery, expression and development of the green fluorescent protein (GFP). Since its discovery, novel fluorescent proteins (FPs) have been engineered to modify their properties at will. The development of mutants able to generate ROS is pursued as a tool in microscopy, optogenetics or PDT. The first successful example was KillerRed, a dimeric protein from the GFP family specifically evolved to efficiently generate ROS.[7] Although it was later shown that KillerRed mainly produces other ROS (superoxide) and not $^1O_2$, it did bring the focus to the potential of FPs as genetically-encoded PSs and has catalyzed the study of ROS photosensitization by FPs at the molecular level. Recently, attention has been turned to flavoproteins. In solution, most flavins are endowed with mild blue fluorescence and undergo intersystem crossing quite efficiently, which in turn is reflected in relatively high yields of $^1O_2$ production. These properties may dramatically change when the flavin is bound to the protein. Rational design of the light, oxygen, voltage (LOV) domain of flavoproteins led to miniSOG, the first flavoprotein succeeding in generating $^1O_2$.[8] A palette of new flavin-binding FPs has been developed and their characterization and *in vitro* application is currently ongoing. Selected candidate proteins are expressed in *E. coli* and cell death successfully induced in a light-dose dependent manner. This has demonstrated that

intracellular generation of $^1O_2$ is sufficient to kill bacteria, which paves the way for the development of novel approaches to overcome antibiotic resistance.

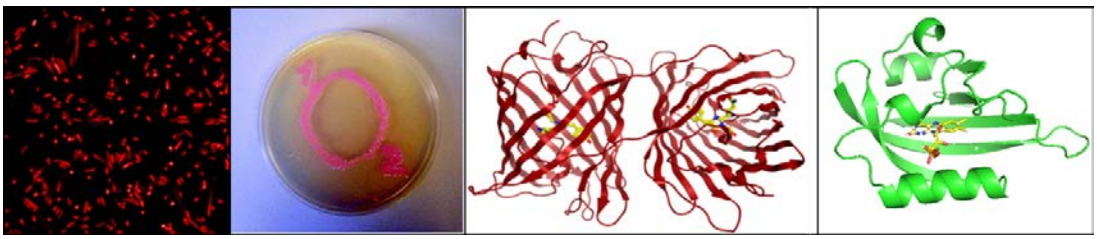

Fig. 2 FP expressed in bacteria and structure of two different FPs.

The beauty of using FPs capable of generating sufficient amounts of $^1O_2$ is multiple. Firstly, we are able to target any protein by fusing our FP-gene to that of the protein of interest. Secondly, the intrinsic fluorescence of the PS makes it a potential theranostic agent. Finally, being able to generate $^1O_2$ opens the door to a plethora of unprecedented opportunities: we can finely tune the amount of $^1O_2$ necessary to provoke cell death, we can damage a fused protein selectively or we can compare the damage at different subcellular locations. The potential of genetically-encoded PSs arises as a powerful tool to be explored and exploited combined with the use of proper carrier systems. But before any of this can happen, improved FPs with enhanced capacity to produce $^1O_2$ needs to be developed.

An old light, still something new under the sun

Despite the promising *in vitro* results, aPDT is not being routinely applied in clinical settings yet. However, clinical studies have been carried out especially in dentistry and dermatology. And it has become a powerful research tool: to help identify the photophysical mechanisms involved in light-mediated cell inactivation to develop potent and clinically compatible PSs; to understand how photoinactivation is affected by key microbial phenotypic elements; to explore novel delivery platforms inspired by current trends in pharmacology and nanotechnology and to identify photoinactivation applications beyond the clinical setting. In this sense, the current technology presents exciting possibilities in its potential medical and even industrial applications, which include decontamination of blood bank supplies, localized elimination of viruses or environmental disinfection. This latest approach is being developed as a tool to prevent malaria and other vector-borne diseases or for environmentally-friendly disinfection of water against microbial and larval pollution, both approaches using sun light.

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