Review Article

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# Light Activated Antibiotics: Do they Exist? A Review

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## ABSTRACT

**Background**: Rampant overuse and misuse of antibiotics has led to a global emergence of bacterial resistance to a plethora of antibiotics. This has thrown a challenge to mankind to devise novel strategies to combat and treat fatal infections which are resistant to these antibiotics. Various novel treatment strategies are being researched in this field. Photodynamic therapy is one such treatment option. Diversification of this Photodynamic Therapy has led to the search of commonly available antibiotics to substitute as photosensitizers. These Light-activated antibiotics can show increased potency at killing microorganisms without leading to bacterial resistance. This review will throw light on some of the commonly used antibiotics that have the potential to be activated by light to produce reactive oxygen species (ROS) which are lethal to bacteria, viruses and fungi.

**Keywords:** Light-Activated Antibiotics, Photodynamic Therapy, Reactive oxygen species, Antimicrobial resistance.

## INTRODUCTION

Infections continue to be the major cause of deaths worldwide.[1] Many factors play an important contributing role such as the emergence of newer diseases, re-emergence of old diseases and very importantly the bacterial resistance to antibiotics.[2] Antibiotics are the most sought form of chemotherapy and have saved millions of lives since its discovery.[3] Antibiotics apart from being used in medicine and surgery is extensively used in non-medical fields such as veterinary sciences, beekeeping, fish farming, food preservation, anti-fouling paints to name a few.[4]

The term 'antibiotic' was coined by Waksman In 1941 to categorize agents exhibiting the property of 'antibiosis.[5] An Antibiotic is a chemical substance produced by microorganisms which have the capacity to inhibit the growth of and even to destroy bacteria or other microorganisms.[6]

With the development of synthetic, semi-synthetic derivatives 'Antimicrobial' replaced the term 'Antibiotic'.[7] An Antimicrobial agent refers to any substance of natural, synthetic or semi-synthetic origin that kills or inhibits the growth of microorganisms, but causes little or no damage to the host.[8]

These antimicrobials include antibiotics, antiseptics, antifungals and antihelminthic. The action of these agents could target specific pathogens or may be effective against a wide range of pathogens.

Evolution of Antibiotics

The antibiotic Era started in late1890s with the discovery of Pyocyanase from the cultivation of Pseudomonas aeruginosa by two Germans Rudolph Emmerich and Oscar Low.[9] In 1909 Salvarasan, an arsenic-based chemical was discovered by Paul Ehrlich that proved to be an effective treatment for syphilis.[10] Paul Ehrlich is considered as 'Father of antimicrobial chemotherapy'.[11] The year 1928 saw a landmark discovery of Penicillin by Alexander Fleming.[12] But it took 12 years for purification of Penicillin for medical use. In 1940 Howard Florey and Ernst Chain devised a purification technique for Pencillin to make it to be used for medicinal

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purpose.[13] This magic bullet was widely used to treat bacterial infections in soldiers during World war II.[12] Alexander Fleming, Ernst Boris Chain and Howard Walter Florey were awarded the Nobel Prize in 1945.

In 1931, Germans Josef Klarer and Fritz Mietzsch at Bayer's discovered Sulfonamidochrysoidine (Prontosil). This Prontosil was a precursor to sulfanilamide. This led to the discovery of sulfonamide group of drugs which had the potential to treat many infections caused by bacteria [9,14] The period from 1950 to 1970 is often considered as the 'Golden age of Antibiotic discovery'. Tetracyclines, Erythromycin, Methicillin, Gentamycin and Vancomycin are some of the notable drugs that were discovered during this period.[13] Last two decades saw very few discoveries of newer novel antibiotics.[15]

Pro and Cons of the Use of Antibiotics

Right from the discovery of Pycocynase, antibiotics still remain the main treatment against fatal bacterial infections.[16]

The Pros of the use of antibiotics are seen with respect to treating life-threatening infections like bacterial meningitis and recurrent urinary tract infections (UTI), Antibiotic prophylaxis in Rheumatic Fever, Recurrent cellulitis and Meningococcal diseases, spontaneous bacterial peritonitis, Surgical site infections(SSIs), transplant recipients or individuals who are immune-suppressed.[17]

The cons of the use of antibiotics is the side effects associated many of the antibiotics that include severe or fatal conditions like hypersensitivity, drug interactions that can either potentiate or reduce the actions of other drugs when taken together, development of opportunistic pathogens and lastly bacterial resistance.[17]

Anti Microbial Resistance (AMR)

Antimicrobial resistance (AMR) is a process wherein a naturally susceptible organism acquires ways of not being affected by an antimicrobial agent.[18] Bacterial resistance to antibiotics is a worldwide challenge associated with high mortality and morbidity. World Health Organization (WHO) has named antibiotic resistance as one of the most important health threats of the 21st century.[19]

Antibiotic resistance crisis has been attributed to a variety of causes. It includes Microbial Causes, Human causes, veterinary medicine, animal farming, and aquaculture.[4]

Microbial causes can be intrinsic resistance, extrinsic resistance or mutational.[20]

Intrinsic resistance

Microorganisms do not have receptors for possible target sites for the drugs. Hence the antimicrobial does not affect them or that it could be that microbes may have additional alteration in their structure thereby causing a low permeability to the antimicrobial agents, e.g. More complex outer layer consisting of LPS makes it difficult for antimicrobials to penetrate a when compared to the gram-positive microorganisms.

Acquired resistance

Sometimes microorganisms acquire ways of not being affected by the antimicrobial agent. This is possible by the change in the genetic composition of the microorganisms.

Microorganisms can pass/exchange genetic material from one to another through a process known as Horizontal Gene Transfer (HGT).

Mechanisms involved in antimicrobial resistance

1. Modification of antibiotic molecule.

The microbes often to cope up with the presence of antimicrobial agent produce enzymes that inactivate the agent.

a. Chemical alteration of the antibiotics.

Both gram-positive and gram-negative microorganisms often produce enzymes which are capable of inducing chemical changes to the antimicrobial molecule

b. Destruction of the antibiotic molecule

Microorganisms produce enzymes that can lead to the inactivation of the antibiotics molecule.

2. Decreased antibiotic penetration and efflux

a. Decreased permeability

The structure of gram-negative microorganism consisting of the outer layer of LPS acts as the first line of defence against the penetration of several antimicrobial agents.

Porins are openings in the cytoplasmic membrane through which antimicrobial agent can gain entry into the bacterial cell. Alterations can occur in the porins and can be attributed to three different processes.

i. The shift in the type of porins expressed
ii. Change in level porins expression
iii. Impairment of porin function

These collectively lead to decreased permeability of the antimicrobial agents and is considered as an important mechanism of resistance.

b. Efflux pumps

Some microorganisms have a specific system called an efflux pump wherein a toxic compound is extruded or pumped out the body of the microorganisms. This affects antimicrobial agents such as Protein synthesis Inhibitors, Fluroquinolones, β-Lactams, Carbapenams, Polymyxins.

3. Change in target sites

Certain microorganisms show resistance to antibiotics by protecting specific targets within it to evade from antibiotics from attaching to it or it can modify the receptors/target site that often results in decreased attachment of antibiotics.

4. Horizontal gene transfer.

Microorganisms can pass/exchange genetic material from one to another. This is known as Horizontal Gene Transfer (HGT).

Three mechanisms are involved in it:

i. Transformation- taking up of free DNA from the environment
ii. Transduction- the movement of genetic material by bacteriophages
iii. Conjugation- transfer of genetic material by direct cell to cell contact or by bridging.[2, 20,21,22]

Human causes

1. Large human population
2. Rampant and injudicious prescription by the general physicians including sub-therapeutic dosing and wrong choice of medicine
3. Medications commonly available over the counter drugs (OTC) as well as self-prescription by the patients
4. Underuse of drugs limiting to the relief in symptoms by the patients
5. Overuse and misuse of antibiotics[23]

Growth promoters in animals

Antibiotics are extensively used in feed or water to accelerate growth in animals. Overuse and sub-inhibitory uses lead to the growth of resistant bacteria which in turn contaminates the environment.[4]

Veterinary medicine and Aquaculture

Just as in humans, antimicrobials are widely used to treat sick animals. But the main concern is chronic use of sub-therapeutic amounts of antibiotics for growth promotion. Another field where antibiotics are extensively used along with feed is fish farming. It is mainly to prevent large scale infections of the fishes. Unhealthy environmental conditions favour the growth of resistant bacteria which in turn can spread to the other farms in proximity through contamination of the groundwater. [24,25,26]

One of the most important mechanisms which often overlooked is the sewage system. This system contains a significant amount of pharmaceutical wastes along with resistant bacteria from the excretes of humans as well as the livestock. These sewage systems are not designed to remove antibiotics and other drugs, thus feeding into natural bodies of water. The levels of antibiotic resistant genes as much as 28000 times are found in animals waste slurries than those in untreated soils. These genes can be passed onto humans through contaminated vegetables. The global death from drug-resistant bacterial infections is projected to increase from 700,000 in 2014 to 10 million by 2050. [27]

With a dearth of new antibiotics under research or under development, and a concomitant microorganisms becoming resistant to older

antibiotics has compromised our ability to treat old or combating new infections. Hence a continued search for novel antimicrobial techniques which will be more efficient, cost-effective, easily available and more importantly doesn't give rise to bacterial resistance.

Photodynamic therapy is one such novel technique that has emerged as an alternative to the use of antibiotics in the management of infections. Photodynamic therapy uses a non-toxic dye or photosensitizer which when activated by light of a suitable wavelength in the presence of molecular oxygen results in the production of highly reactive oxygen species (ROS) which have toxic bactericidal effect without any harm to the host.[28, 29]

History of Photodynamic therapy

Light has been used in therapeutic form since ages in ancient civilizations like India, Egypt to treat a variety of diseases ranging from skin diseases to rickets.[30]

In 1900, Oscar Raab, a medical student under Professor Herman Von Tappeiner, discovered that cells of Paramecium infusoria were killed when exposed to Acridine dye and light. Three years later, Von Tappeiner and Jodblauer noted molecular oxygen was a key essential component required to kill protozoa when used with the combination of aniline dye and light. Hence the term 'Photodynamic Therapy' was coined by Herman Von Tappeiner. In 1905, Von Tappeiner, along with Jesionek, a dermatologist successfully treated a variety of skin disorders by using 5% Eosin dye along with artificial light sources.

Hausmann in 1911 reported the use of Hematoporphyrin as a photosensitizer. Auler and Banzer continued with the use of Hematoporphyrin to treat skin disorders which was confirmed by further studies by Figge.

Dougherty in 1978 made a great discovery with a new photosensitizer which was called as Hematoporphyrin purified derivative (HPD) and used it extensively to treat a variety of Oncologic and Non- oncologic cases. Another breakthrough was done by Kennedy in 1990 which the discovery of 5- Aminolevulinic acid (ALA) which is now widely used in field of Dermatology.[31-34]

Originally Photodynamic therapy was directed at treating localized infections. Now it is widely used in various conditions like Oncologic and Non-oncologic dermatological conditions, Neoplastic conditions, Inflammatory/ Immune disorders and infectious diseases.[35-38]

Light activated Antibiotics (LAAs)

One of the inherent drawbacks of a conventional Photodynamic Therapy is in the management of localized infections like periodontal diseases. Once the light is stopped or switched off, the photochemical production of highly reactive oxygen species (ROS) ceases and often there is a tendency of regrowth of the microorganisms in that area leading to re-infection. This is where the current research is directed at, to discover newer agents that would continue to exert their action for a longer duration of time. Light-activated antibiotics are one of the better alternatives to substitute conventional photosensitizes in a PDT.

Light-activated antibiotics (LAAs) is a type of PDT strategy wherein certain antibiotics are potentiated via the light of suitable wavelength. Upon activation by light of a suitable wavelength, the Light activated antibiotic generates reactive oxygen species which are extremely detrimental to a wide range of microorganisms including bacteria, viruses and fungi.

The advantages are twofold, firstly efficient bactericidal effect even with a low dosage of antibiotics, secondly, there is a continuous presence of antibiotic residue even after the action of PDT ceases.[39]

Mechanism of action of LAAs

On illumination, a cascade of photochemical events occur. The first step is the absorption of light by the LAAs, which leads to a photon being absorbed by the LAAs, producing the first excited state (1LAA*). This 1LAA* has a very short lifetime ranging from few nano sec to pico secs and this high energy gets dissipated through fluorescence and internal conversion. Alternatively a part of this 1LAA* gets converted into an excited triplet state by interstate crossover process. The molecules in the excited triplet state (3LAAs*) have much longer lifetime ranging from micro to milli secs and undergoes physical and chemical reactions by two different

pathways to produce ROS which are detrimental to the infecting agents.

The type I reaction occurs through electron or hydrogen transfer directly from the LAAs by directly reacting with a substrate often water to produce radical ions such as superoxide anion(-$O_2$) which then reacts with molecular oxygen to produce highly reactive species such as Hydroxyl radical and Hydrogen peroxide. Type II reaction involves energy transfer from the excited state of LAAs interacting with the ground state molecular oxygen to produce highly reactive singlet oxygen (SO-) which can cause rapid cytotoxic effects and cause damage to cell wall as well as to the cytoplasmic membrane of bacteria, viruses and fungi. (Figure 1)

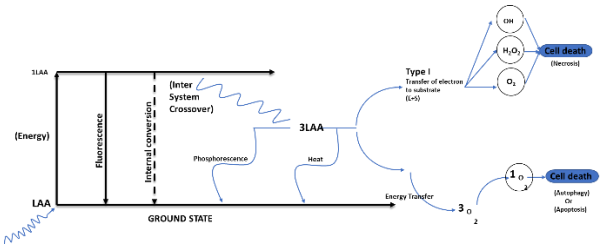


Fig 1: Mechanism of action of LAA

All the ROS ($^1O_2$, $O_2$, $H_2O_2$ and H0) produced through these pathways have a broad spectrum of activity and can destroy a variety of bacterial biological molecules like proteins, lipids and nucleic acid. Of these ROS HO and $^1O_2$* have the most powerful microbicidal activity.[40-48]

Both Type, I and Type II, requires molecular oxygen for eliciting an antimicrobial action (oxygen-dependent process).

Recently Type III an Oxygen Independent photoactivation of the microbes was proposed by Micheal Hamblin et.al. LAAs like Tetracyclines rely upon specific binding between the agent and a defined molecular structure like one or more protein of 30s ribosomal subunit. Once the agent interacts with the receptor and on activation by light of specific wavelength can initiate a photochemical reaction leading to formation or breakage of covalent bonds formed often termed as ' photo adducts'. This reaction can occur mostly through an oxygen-independent mechanism but can interact with oxygen to proceed as oxygen-dependent mechanism.[49]

The main advantages of LAAs over conventional photosensitizers would be that LAAs are commonly available and are very cost-effective, the light sources used to activate the LAAs increases the potency of the destruction of the microorganisms including the resistant ones. LAA are time tested antibiotics and as per se has minimal side effects. Since the LAAs are used topically, it provides a rapid, efficient and biocompatible way of bacterial killing. This procedure also does not lead to the development of resistant microorganisms.[39]

Studies on Different Light-Activated Antibiotics

Since PDT is a novel method of bacterial killing, various antibiotics are researched for their 'Dual Action.'

Ernesto[50] et al. in 1979 did a study to evaluate the effect of photo dynamically activated tetracyclines on animal viruses. The results demonstrated that tetracycline derivatives like Demclochlortetracycline, Chlortetracycline and Doxycycline were found to be efficient in inactivating animal viruses like Venezuelan Equine encephalitis (VEE) viruses. The mechanism behind this inactivation is not completely understood, but the site of action may be located to the interior of the virus capsid at the level of the nucleic acid. The damage elicited by the DMCT- mediated photodynamic inactivation does not occur through the RNA but rather by mutagenesis by changes in the specific nucleotide residues.

In 1961 Martin[51] et al. conducted a study to examine the involvement of oxygen radicals in tetracycline toxicity and the response of E-coli B to tetracyclines phototoxicity in vivo. The results revealed superoxide, hydrogen peroxide and Hydroxyl radicals are generated by illuminated tetracyclines and are molecular agents of tetracycline phototoxicity in E. coli B. The possible mechanisms may be when tetracyclines are irradiated with UVA generates $O_2$- and $H_2O_2$ is generated by $O_2$- dismutation. Photolytic deamination is the first step in the degradation of UVA illuminated Tetracyclines. Subsequently, it can involve the formation of $O_2$- radicals by oxidation. Both $O_2$- and $H_2O_2$ are capable of causing bacterial cell damage.

Weijun Xuan[52] et al. in 2018 investigated whether tetracyclines could function as dual-action light

activated antimicrobial compound using UVA and blue light and also if the addition of potassium iodide could potentiate the PDI effect on tetracyclines. The result revealed that Demeclocycline and Doxycycline are dual antibacterial compound functioning as antibiotics in the dark and as PS under activation with blue or UVA light. The photochemical mechanisms underlying the use of these are complex and include Type II generation of Singlet O2. Type I generation of radicals intermediates and a direct O2 dependent photoaffinity labelling of ribosomes occur. The potency of the destruction of microorganism can be increased by 200-400Mm by addition of KI.

Ya He[53] et al. in 2018 studied the effect of photoactivated tetracyclines on gram-positive (Methicillin-resistant Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria. The results showed that tetracyclines accumulated in bacterial ribosomes where they could be activated with UVA/Blue light leading to the production of ROS, which is bacteriocidal.

Tetracyclines have many advantages when used as LAA as compared to a conventional PS. Firstly Tetracyclines are taken up into Gram-Negative cells vis the OmpF and OmpC porin channels. Once inside the periplasm, tetracyclines can easily diffuse through the lipid bilayer of the cytoplasmic membrane to target the ribosomes. There is the formation of Covalent bonding with the ribosomes, which are termed 'Photoadducts'. This ensures direct and permanent photochemical damage to the bacterial ribosomes. Another advantage of using Tetracyclines is that of the continued presence of the antibiotic effect of non-degraded tetracyclines exerting a biologically active preventing bacterial regrowth in the tissue even after the cessation of the light.[54]

Qi Jiang[39] in 2020 investigated the ROS generating ability of six types of antibiotics like PolymyxinB, Sulfamethizole, Norfloxacin, Teicoplanin, Ampicillin, Kanamycin. The results revealed that Newer light-activated antibiotics included Polymyxin B, Sulphamethizole, Norfloxacin and Teicoplanin. These antibiotics are capable of producing ROS under light irradiation suggesting that these can be used as PS for the photodynamic killing of microorganisms when compared to general PS, these antibiotics showed more potency because of their intrinsic anti bacterial properties and specific binding with the microorganisms.

The studies based on various dual light-activated antibiotics show great promise for local use of these agents in the management of localized and life-threatening infections. Further, it can be an effective weapon to overcome the global threat of bacterial resistance. More large scale and exhaustive research are the need of the hour if mankind can ever win the battle against microorganisms.

## CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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