Acta Scientific Pharmaceutical Sciences (ISSN: 2581-5423)

Volume 8 Issue 4 April 2024

Review Article

A Beacon of Hope in the Battle Against Antimicrobial Resistance

Pavan Kalyan B[1], Diana Nahakpam[1], DK Brahma[2]* and Rajesh Kumar M[1]

[1]*PGTs of Department of Pharmacology, NEIGRIHMS, Shillong, India*

[2]*Additional Professor, Department of Pharmacology, NEIGRIHMS, Shillong, India*

***Corresponding Author:** DK Brahma, Additional Professor, Department of Pharmacology, NEIGRIHMS, Shillong, India.

DOI: 10.31080/ASPS.2024.08.1049

Received: March 01, 2024

Published: March 06, 2024

Abstract

The advent of antibiotics during the 1940s revolutionized medicine, reducing mortality rates from infectious diseases. However, the emergence of antibiotic resistance presents a formidable global health threat, urging the search for new antimicrobial agents. The pipeline of new antibiotic research and development at present is very narrow and only a few antibiotics have been marketed over the last decades and they often resemble older, already known antibiotics. Traditional methods of antibiotic discovery have faced diminishing returns, leading to a shift towards target-based strategies, which have struggled to yield novel antibiotics. The recent discovery of clovibactin from previously uncultured soil bacteria represents a promising breakthrough in this arena. It exhibits potent bactericidal activity against a wide range of Gram-positive pathogens, including multidrug-resistant strains, with a distinctive mechanism of action targeting bacterial cell wall precursors. Additionally, it has demonstrated favorable pharmacokinetic properties and efficacy in animal models. Despite its potential, further investigation is necessary to evaluate its safety and clinical efficacy in the human population. Clovibactin brings a glimmer of hope in the ongoing battle against antibiotic resistance. Clovibactin is a promising discovery in fighting antibiotic-resistant pathogens and enhances its clinical usefulness.

Keywords: Clovibactin; Antibiotic Resistance; Gram-Positive Pathogens

Introduction

Antibiotics have been revolutionary in medicine, fundamentally changing how we combat infections and prolonging our lifespan. Before their discovery, over half of all deaths were due to infections. However, antibiotics drastically reduced this mortality rate and allowed for significant advancements in medical procedures, by effectively controlling infections. Additionally, antibiotics have also played a crucial role in preventing disease transmission and promoting public health [1].

Despite their benefits, antibiotics are not without challenges. One major issue is the development of antibiotic resistance, which can render these drugs ineffective over time. Efforts to address this problem, including antibiotic stewardship programs and restrictions on their use in agriculture, have been implemented. However, the pace of antibiotic discovery and development has slowed down significantly in recent decades, raising concerns about the emergence of a "post-antibiotic era". Some highly resistant strains of bacteria, like pandrug-resistant Acinetobacter baumannii and Mycobacterium tuberculosis, have already become untreatable with existing antibiotics, highlighting the urgency of finding new solutions to combat infectious diseases [1].

The 1980s and 1990s marked a shift away from the traditional method of discovering antibiotics, known as the Waksman platform, which relied on a cell death phenotype-based approach pioneered by Selman Waksman. This approach had been highly successful during the "Golden Era" of antibiotic discovery, yielding many of the antibiotic chemical structures still in use today. However, as time passed, this method began to produce diminishing returns, as environmental microbes, the primary source of these antibiotics, increasingly yielded known compounds. Consequently,

pharmaceutical companies turned to more target-based strategies, leveraging advancements in genomics and technology to identify new drug candidates [1].

While this approach proved effective in other areas of drug discovery, it struggled to produce new antibiotics. The pharmaceutical sector's experience with target-based antibiotic discovery was not very successful and led to high costs and poor returns on investment. As a result, many large pharmaceutical companies withdrew from antibiotic research, leaving smaller companies to fill the void [1].

Currently, there is no new antibiotic in development, which is concerning. Even as antibiotic resistance continues to rise, with a significant public health threat. Efforts to preserve existing antibiotics, such as the World Health Organization's AWaRe Classification Database, are important but further diminish the economic prospects of antibiotic discovery [1].

A hopeful development has been made by scientists in the fight against drug-resistant bacteria in recent times with the discovery of Clovibactin, a novel antibiotic discovered from bacteria that were previously thought to be unconquerable, i.e. isolated from uncultured soil bacteria. Scientists have observed the amazing effectiveness of clovibactin in eliminating drug-resistant Gram-positive bacterial infections without detectable resistance development which provides a glimmer of hope in our struggle against dangerous bacteria and multi-resistant "superbugs".

Discovery of clovibactin

Certain environmental bacteria and spores may require extended incubation periods to initiate growth in laboratory conditions [2]. This prolonged incubation allows access to microorganisms that would otherwise be missed using standard cultivation techniques. To promote the growth of spore-forming actinomycetes, soil samples were initially incubated at 65°C for 30 minutes. The soil was then diluted and plated onto microtiter plates, with each well, containing no more than one bacterial cell. Growth was monitored over several weeks, and colonies were sub-cultured and screened for antimicrobial activity against Staphylococcus aureus. One particularly promising producer, identified as Eleftheria terrae ssp. carolina, was detected after 12 weeks of incubation.

The antimicrobial compound produced by *E. terrae* ssp. carolina was subsequently investigated. Initial attempts led to the identification of kalimantacin, but further analysis revealed activity against Bacillus subtilis, which is not typical for kalimantacin [3]. Whole genome sequencing revealed a biosynthetic gene cluster (BGC) similar to the kalimantacin/batumin operon. By disrupting a key gene within this operon, bat1, kalimantacin production was significantly reduced by using homologous recombination of a suicide vector [4].

Further isolation and characterization of the fermentation broth by separation using HPLC and Bioassay-guided isolation led to the discovery of a novel depsipeptide compound, which was named as clovibactin. Structural analysis confirmed its uniqueness and revealed similarities to the antibiotic teixobactin, although clovibactin possesses distinct features, including a shorter linear N-terminus and different amino acid residues [5].

The genome of *E. terrae* ssp. carolina was extensively analyzed using PacBio, revealing a total of 19 predicted BGCs, with 14 containing non-ribosomal peptide synthetase (NRPS)-like elements. The gene cluster responsible for clovibactin biosynthesis was identified and consists of two NRPS genes (cloA and cloB), a transporter gene (cloC), and a tailoring enzyme (cloD) [5].

The biosynthetic gene cluster responsible for clovibactin production was identified using antiSMASH version 5.1.1. This cluster comprises two NRPS genes (cloA and cloB), a transporter gene (cloC), and a tailoring enzyme (cloD) [6].

The identification of clovibactin from this strain emphasises the potential of investigating previously uncultured bacteria as a valuable reservoir of novel bioactive compounds, especially in the realm of antibiotic discovery.

Clovibactin exhibits bactericidal activity, with minimal bactericidal activity (MBC) against *S. aureus* at twice the minimum inhibitory concentration (MIC). Further investigation into the time-dependent killing dynamics revealed that clovibactin is more effective at killing S. aureus compared to vancomycin, a commonly used antibiotic. Interestingly, clovibactin also induces strong lysis of bacterial cells, a phenomenon quantified and observed to be more pronounced than with teixobactin, another potent antibiotic known to induce lysis through the action of AtlA, a major cell wall autolysin of *S. aureus* [5].

The mechanism of action of Clovibactin is slightly peculiar. Clovibactin has strong antimicrobial activity and a strong ability to induce cell lysis but it does not exhibit rapid membrane disruption or pore formation. It also does not display any cytotoxic effects against mammalian cells at the highest tested concentration [5].

According to the study by Shukla., *et al*. when In Vivo studies were conducted to evaluate the pharmacokinetic profile of Clovibactin, by administering the drug intravenously at a dose of 20mg/kg in mice, clovibactin was well tolerated. The plasma levels were determined using LC-MSMS and the pharmacokinetic properties were determined using Watson LIMS software. Further evaluation for the efficacy of clovibactin was assessed in a neutropenic mouse thigh infection model involving S. aureus whose immune response of mice was suppressed using cyclophosphamide, allowing for the evaluation of antibiotics in controlling infection without immune assistance. Clovibactin showed antimicrobial effectiveness similar to that of vancomycin [5].

Clovibactin demonstrated an antimicrobial spectrum of activity against a broad range of Gram-positive pathogens, including methicillin-resistant *S. aureus* (MRSA), daptomycin-resistant as well as vancomycin-intermediate resistant (VISA) strains, and also difficult-to-treat vancomycin-resistant *Enterococcus faecalis* and *E. faecium* (VRE). However, it has shown weak activity against *E. coli* [7].

Mechanism of action

According to the study by Shukla., *et al*. clovibactin demonstrated its anti-microbial activity by interfering with the cell wall biosynthesis. It was found that Clovibactin exhibited dose-dependent inhibition of all cell wall biosynthesis reactions utilizing lipid I, lipid II, lipid III_{WTA}, or undecaprenyl-pyrophosphate ($C_{55}PP$) as substrates, suggesting that clovibactin binds to these lipid intermediates rather than directly inhibiting enzyme function [5].

What makes Clovibactin special is that, firstly, clovibactin stands out due to its unique structural characteristics and secondly, In contrast to conventional antibiotics that typically target variable components of bacterial cell wall precursors, clovibactin specifically interacts with the unchanging pyrophosphate moiety present in various bacterial cell wall precursors like $C_{55}PP$, lipid II, and lipid III_{WTA}. These unique characteristics of Clovibactin make it stand out from traditional antibiotic mechanisms [5].

Conclusion

For a long time, antibiotics have served as crucial tools in pharmacotherapy for treating patients. However, the emergence of antimicrobial resistance presents a substantial global threat to human health. According to a study by The Lancet in 2019, as many as an estimated 4.95 million deaths were attributed to antimicrobial resistance [8]. In the current challenging era, where the discovery of new antimicrobials has taken a backseat, the emergence of Clovibactin offers a beacon of hope in the ongoing battle against antimicrobial resistance.

Clovibactin exhibits a broad spectrum of activity against Gram-positive pathogens, making it particularly noteworthy. It has shown the ability to effectively target a diverse array of bacterial strains, particularly its effect against Staphylococcus aureus. Furthermore, it has also shown the ability to target multiple essential cell wall precursors. Additionally, clovibactin's selective binding and ability to avoid resistance mechanisms contribute to its potent antibacterial efficacy. All these unique characteristics make Clovibactin a promising discovery in fighting antibiotic-resistant pathogens and enhance its clinical usefulness.

While this has been a difficult task, the discovery of clovibactin has definitely renewed hope that we can fight our way out of this antibiotic resistance crisis. However, an unfortunate, yet common, example is that antibiotics that appear safe in animals may turn out to be toxic at the higher doses required to treat humans. Such unforeseen complications during the development phase are one part of the reason why more than 98.5% of newly discovered antibiotics never make it out of the lab. It is too early to comment on whether clovibactin is the savior we've been longing for.

Bibliography

1. Cook MA and Wright GD. "The past, present, and future of antibiotics". *Science Translational Medicine* 14.657 (2022): eabo7793.

2. Buerger S., *et al*. "Microbial scout hypothesis and microbial discovery". *Applied and Environmental Microbiology* 78.9 (2012): 3229-3233.

3. Fage CD., *et al*. "The Kalimantacin Polyketide Antibiotics Inhibit Fatty Acid Biosynthesis in Staphylococcus aureus by Targeting the Enoyl-Acyl Carrier Protein Binding Site of FabI". *Angewandte Chemie International Edition* 59.26 (2020): 10549-10556.

4. Fernández-Martínez L and Bibb M. "Use of the Meganuclease I-SceI of Saccharomycescerevisiae to select for gene deletions in actinomycetes". *Scientific Report* 4 (2014): 7100.

5. Shukla R., *et al*. "A new antibiotic from an uncultured bacterium binds to an immutable target". *bioRxiv* (2023).

6. Hsu ST., *et al*. "The nisin–lipid II complex reveals a pyrophosphate cage that provides a blueprint for novel antibiotics". *Nature Structural and Molecular Biology* 11 (2004): 963-967.

7. Lebreton F., *et al*. "Tracing the Enterococci from Paleozoic Origins to the Hospital". *Cell* 169 (2017): 849-861e813.

8. Antimicrobial Resistance Collaborators. "Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis". *Lancet* 399 (2022): 629-655.