

Directions in the development of modern and promising antimicrobial agents

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The use of antibiotics is becoming increasingly limited. This is mainly due to the development of resistance to pathogenic bacteria, and, over time, more and more bacteria will become resistant to antimicrobials. This problem inevitably leads to the conclusion that studies into alternative methods of combating pathogens, which are necessary to develop sufficiently reliable and effective therapies for bacterial infections, are indispensable. This review highlights some recent developments in conventional antibiotic and non-antibiotic treatment strategies. It has been shown that traditional antibacterial targets include derivatives of known antibiotic classes, new chemical classes with new targets, as well as unknown or undefined agents with unclear targets. Promising strategies for combating microbial pathogens have been identified, including new targets, namely, toxin secretion systems, biofilm formation, and adhesion mechanisms that affect quorum sensing of microbial populations. In addition, it is important to use new antimicrobial agents with other, non-antibiotic, mechanisms of action: phage and phage-derived peptides, microbiota-modulating therapies, and enhancing immune response.

Keywords: conventional antibiotic targets, nonantibiotic targets, nonantibiotic treatment, infections, resistance

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INTRODUCTION

The advent of antibiotics in the first half of the twentieth century catalysed a medical revolution by drastically reducing mortality from bacterial infections. Antibiotics contributed significantly to an extension in the average life expectancy in the USA from 59.7 years in 1930 to 78.7 years in 2010 (Arias et al., 2016), providing clinicians with a wide range of tools to prevent and fight bacterial infections. The mechanisms of action of these substances are mainly related to blocking the development and reproduction of bacterial cells, which leads to the disruption of the synthesis of their cell wall and cytoplasmic membrane. Very quickly, however, due to the high adaptability of pathogens and the acquisition of resistance, these substances lose their relevance as antimicrobial substances. To solve this problem, the existing classes of antibiotics with limited cross-resistance to existing drugs have been modified and new classes of antibiotics have been introduced (Aminov, 2010).

However, the result of uncontrolled or widespread use of antimicrobials is microbial resistance to the use of each new drug, and the proliferation of resistant pathogens is becoming a major problem (Palumbi, 2001; Tsiodras et al., 2001; Lewis et al., 2005; Clatworthy et al., 2007; Gentry et al., 2008; Morgan et al., 2011). Moreover, due to the high doses of antibiotics required to trigger efflux mechanisms in gram-negative bacteria, intoxication of the body can occur (Baker et al., 2018). This threatens with the situation when microbial pathogens do not respond to the existing antibiotics, and therefore there is a need to develop new effective strategies for treating infectious diseases with substances of a different nature. These methods include treatment aimed at modeling the microbiota, namely, the use of antibacterial vaccines and antibodies; phages or phage-derived proteins, antiviral agents, potentiators that enhance, augment, or transform other agents, and the use of immunomodulators developed to treat bacterial diseases (Tenson, Mankin, 2006; Motley, Fries, 2017; Theuretzbacher, Piddock, 2019; Kortright et al.,

2019; Miró-Canturri, 2019; Rello et al., 2019; Monserrat-Martinez et al., 2019; Vaccines to tackle drug resistant infections 2019).

In this review, we explain some traditional targets to fight given the development of antibiotic resistance of bacteria, and discuss alternative ways to control microbial pathogens.

The main effect of antibiotics is achieved through direct destruction of the pathogenic flora. The vast majority of antibacterial molecules are artificially synthesised; they are not natural compounds. Their registration by regulatory authorities occurs according to simple and well-known algorithms. The development of this trend in pharmacology takes place in several directions: derivatisation of well-studied molecules; research into new known molecules with new target organisms and into innovative unexplored molecules with undefined targets.

DISCUSSION

Derivatives of the classes of known antibiotics. Most modern antibiotics are derivatives of natural substances that have been used for many years (Fernebrot, 2011). The development of resistance of pathogens to antibiotics can be slowed down by modifying known and already used substances. Such modification may include increased intermolecular and intramolecular interactions and is considered an effective way to combat resistant forms of pathogens (Fernebrot, 2011; Silver, 2007). For example, vancomycin could interrupt bacterial cell wall synthesis by binding with high proximity to peptidoglycan and preventing cell wall assembly (Perkins, 1982; Romaniuk, Cegelski, 2015). The emergence of vancomycin-resistant enterococci (VRE), which was associated with their ability to disrupt the factors of interaction with the antibiotic (repeats d-Ala-d-Ala), was recorded after several decades of use of this antibiotic (Noble et al., 1992; Murray, 2000; McKessar et al., 2000). Modified vancomycin was developed to avoid this ability of pathogenic enterococci (Xie et al., 2011; Okano et al., 2017), but this did not stop the growth of bacteria and they soon became resistant again.

New targets for new chemical groups. New target molecules contain new sites for interactions in the area of ribosomes, membranes, gene interference, and metabolism (Ma et al., 2016; Silver, 2016; Xie et al., 2018; Ciumac et al., 2019). Some of these approaches have already been studied, but clinical development has not begun.

Unknown or undefined agents with unclear targets. This group includes several basic groups of antimicrobial molecules depending on their origin and structure: synthetic or natural antimicrobial peptides and proteins (AMPs), natural products, and UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC) inhibitors. AMP is a well-studied natural compound with clear antibacterial effects. These molecules are the basis for the synthesis of peptides and peptide-like molecules (Kang et al., 2017; Molchanova et al., 2017; Moravej et al., 2018; Torres et al., 2019). The main goals for research in this direction consist in reducing the cost of production of the substances, reducing the impact of proteolytic degradation to increase the half-life *in vivo*, and improving safety (Kuppusamy et al., 2019). The basis of the modern market for antibiotics is precisely such natural substances. Developments in the field of genomics and chemical technologies enable increasing the effectiveness of research in the field of pharmacology of natural molecules (Fedorenko et al., 2015; Wright, 2017; Rossiter et al., 2017). LpxC inhibitors targeting the first stage of lipid A production have been studied exclusively prior to phase I clinical trials. This is due to toxicity, which was identified in a mouse model. In addition, significant local inflammatory reactions were noted after injections (Erwin, 2016; Liu, Ma, 2018; Cohen et al., 2019). The toxicity of the molecules used to inhibit LpxC is the main obstacle to the development of this direction. A growing body of knowledge in this area, such as recent CARB-X-supported studies related to LpxC inhibition (the Shared Platform for Antibiotic Research and Knowledge (SPARK), Pew Trusts), may provide an impetus to overcome some of the barriers to the use of this group of

antibiotics. This instance highlights the general difficulties associated with toxicity in transferring molecular safety models from preclinical trials to clinical use.

New nonantibiotic target. In this section, we present an overview of several ‘nonantibiotic’ approaches aimed at the treatment and prevention of bacterial infections. When bacteria-caused infections occur, pathogens produce virulence factors. The molecules allow pathogens to resist the body’s self-cleansing, gain access to deeper tissues, penetrate them, and damage host cells. For the productive treatment of bacterial infections, agents have been developed that block the activity of virulence factors and therefore halt pathogenesis until the host’s immune response limits or kills the bacteria.

Targeting toxins and secretion systems. Secreted toxins play a significant role in the pathogenesis of many medically necessary bacteria, and several of these have been targeted with the aim of blocking infection. Usually, antibodies and antibody-drug conjugates are the inhibitors of a toxin. Currently, only three antibodies against bacterial infections have been approved for use (Lowy et al., 2010; López et al., 2015; Wilcox et al., 2017). Their main effect is the neutralisation of toxins that affect virulence and, consequently, the pathological process. To date, studies into the antibodies with multiple virulence determinants have not yielded positive results.

Some of the antibody problems described also arise in the development of vaccines against multi-resistant pathogens. The lack of preclinical predictive models has led to the failure of a number of bacterial vaccines in late stages of clinical trials. The absence of such models may lead to poor vaccine efficacy in the future (Priebe, Goldberg, 2014; Redi et al., 2018). Published studies on the evaluation of promising vaccines showed the inconsistency of the target pathogens used for development or the uncertainty of development results (<https://vaccinesforamr.org>). In order to affect human cells, toxins must overcome the membranes of the bacterial cell, since they are synthesised directly in the bacterial cytosol. A number of

specific secretory systems acquired by bacteria are associated with this. These systems can be used as targets for pharmaceuticals. An example is type III secretion systems (T3SSs) inherent in gram-negative bacteria. Type III secretion (T3S) is a multiprotein needle apparatus with which the bacterium can inject the produced compounds directly into the cells of the host. It is known that T3S of different types of bacteria are similar to each other, despite differences in the secreted toxins. This leads to the conclusion that T3S inhibitors can be used to combat a wide range of pathogens (Swietnicki et al., 2011).

Targeting biofilms and adherence. Biofilms that form on inert surfaces such as catheters, prosthetic joints, or heart valves can interfere with the penetration of antibiotics and are major sources of infection (Kester, Fortune, 2013; Ribeiro et al., 2016). To prevent the occurrence of microbial infections both in the biofilm and in the planktonic phase of growth, targeting the adhesins is used. With the help of such adhesins the pathogen is fixed on the mucosal surface (Krachler, Orth, 2013). In the process of infection, microbial pathogens change the internal environment of the host cell by attaching to cellular receptors of their own proteins, which leads to the disruption of the structure of cell membranes and stimulation of various intracellular processes (Shaw et al., 2001; Ide et al., 2001; Pinchuk et al., 2010; Wilson, 2014; Jimenez et al., 2016; Gilbert et al., 2017). One of these processes is the inhibition of the activity of the membrane enzyme sortase A, which is a determining factor in the interaction of antibiotics with the peptidoglycan of the cell wall of gram-positive pathogens (Mazmanian et al., 2001; Ton-That, Schneewind, 2004; Zhang et al., 2014; Thappeta et al., 2020).

Quorum sensing. Quorum sensing is one of the leading mechanisms of microorganism interaction within the population thanks to which they can sense the influence of the external environment as a single organism and produce a collective response. Such a response is often the activation or expression of genes, which depends on the concentration of autoin-

ducers, hormone-like molecules that are synthesised because of intercellular interactions (Vasil, 2003; Waters, Bassler, 2005).

The critical concentration of autoinducers is a signal to the microorganism population to suppress the manufacture of its own virulence factors in order to conceal and postpone the host immune system's reaction. (Deep, 2011). The restoration of the biosynthesis of substances responsible for the pathogenesis of inflammation occurs after the accumulation of a high concentration of pathogens that can neutralize protective mechanisms, and thus the inflammatory process begins.

In the regulation of the synthesis of virulence factors by pathogens, their formation in the form of a biofilm also plays a role.

Among other factors, the functioning of the quorum sensing (QS) system is ensured by the biosynthesis of peptides that are specific to each microorganism, so they can be effective targets for the action of new antiseptics. For example, gram-negative bacteria are dependent on acyl-homoserine lactones (AHL) (Pearson et al., 1994; Whitehead et al., 2001; Njoroge, Sperandio, 2009), the precursor of which is S-adenosylmethionine (SAM) (Papenfort, Bassler, 2016), and oligopeptides are the signaling compounds for the QS system of gram-positive bacteria. The latter have an individual sequence for each species of bacteria, and their synthesis is genetically determined (Ng, Bassler, 2009).

However, the protein nature of these peptides (AHL, SAM) allows the use of appropriate enzymes (lactonases, acylases, or oxidoreductases) for their degradation, and thus inactivation of QS signals of pathogens and its inhibition (Leadbetter, Greenberg, 2000; Dong et al., 2000; Dong et al., 2001; Uroz et al., 2005; LaSarre, Federle, 2013). The possibility of non-enzymatic inactivation of these peptides is also being studied. Another approach is based on blocking autoinducers of pathogens to deprive them of the opportunity to disguise themselves and become immediately 'visible' to the host's immune system. By removing or inactivating autoinducers, it is possible to achieve high

specificity of action of such antimicrobial drugs, which will also minimise the development of pathogen resistance.

Phage and phage-derived peptides. Bacteriophages were used for antibacterial therapy before the advent of antibiotics, and the rise in infections caused by multidrug-resistant (MDR) strains of bacteria has sparked renewed interest in this therapy (Knoll, Mylonakis, 2014). Compared to traditional antibiotics, phages have important advantages: they do not infect human cells and practically do not affect the normal microbial flora (Rohde et al., 2018; Kortright et al., 2019; McCallin et al., 2019). In the past, phages were used in preparations only for external use (Morozova et al., 2018); currently, preparations for intravenous administration and aerosols are being developed (Chang et al., 2018).

To improve phage therapy, phages are developed to increase their infectivity and host range, and individual phage components are purified to target bacteria (Nobrega et al., 2015). Particular attention should be paid to endolysins, which are obtained from phages (Fischetti, 2018; Abdelkader et al., 2019). This group of substances is characterised by a highly specific bacteriolytic effect that occurs upon direct contact. There are reports of the development of drugs against *Staphylococcus aureus* infections based on endolysins (Gentry et al., 2008). Molecular methods, namely protein engineering, lead to the emergence of new methods of combating gram-negative microorganisms (Oliveira et al., 2018), although such projects are not numerous and require in-depth study (Briers, Lavigne, 2015).

Microbiota-modulating therapies. Over the past decades, we have become increasingly aware of the importance of the microbiome for human health and disease. A link has been established between the human microbiota and disease condition, including diabetes, cardiovascular disease, and even mental health. The main target of preclinical studies is *Clostridium difficile*, a representative of the intestinal microbiota. There is practically no mention of the microbiota from the lungs,

sinuses, or the skin. The results of the study of metagenomic, computational, and synthetic biology helped to renew the interest of researchers in the human microbiota (Falony et al., 2019). In order to reduce the occurrence of *C. difficile* relapses and to correct other disorders, a microbiota modification was used to study and test its effectiveness (Camarota et al., 2017; Iqbal et al., 2018; Keller-mayer, 2019). The accuracy of genetic engineering using known probiotics is improved by such approaches (Waters, Bassler, 2005). Thus, it is possible to increase the expression of specific antimicrobials (Fehér et al., 2017; Ozdemir et al., 2018; Ghosh et al., 2019) and at the same time reduce the likelihood of transfer of potential pathogens (Ramachandran, Bikard, 2019).

Modulation of the immune response. The ability to adjust the mechanisms of the host's immune response to infection can be considered one of the important methods of combating pathogens that will prevent the spread of infection in the early stage. Directing macrophages to the site of infection and enhancing the immune response may eliminate the need for antibiotics altogether.

A number of authors demonstrated the role of antimicrobial peptides and proteins in eukaryotic and prokaryotic organisms in the formation of the immune response at the first line of infection (Zasloff, 2002; Ganz, 2003; Reddy et al., 2004; Bahar, Ren, 2013). Containing up to 60 amino acids, these molecules, act as antibiotics under physiological conditions and their antimicrobial activity is mainly due to membrane-lytic action. However, the concomitant effect of AMPs has been established, which consist in influencing the processes of cytokine activity, chemotaxis, antigen detection (Territo et al., 1989; Yang et al., 2000; Niyonsaba et al., 2002; Yang et al., 2002; Kurosaka et al., 2005), increased protection and inhibition of the infectious process, as well as wound healing (Heilborn et al., 2003; Tokumaru et al., 2005; Baroni et al., 2009), complementing the bactericidal effect of other antimicrobials (Lai, Gallo, 2009).

The latest research has developed vitamin-based nanoparticles. These nanoparticles are particularly good at delivering messenger RNA, while constructing messenger RNA encoding antimicrobial peptides and signal proteins and transporting them to macrophages produced by donor monocytes in the cell. Since macrophages naturally have antibacterial activity, adding other antibacterial peptides to the cells facilitates further enhancement of the antibacterial activity and helps the macrophages eliminate bacteria (Hou et al., 2020).

Classical antibacterial drugs act on target organisms by altering cellular processes. As a result of many years of treatment of the population with high doses of antibiotics and their misuse, we have entered the 'postantibiotic era'. Many microorganisms have become resistant to a wide range of antibacterial drugs. New, modern antibiotics are being developed, but the same problem arises: the body is able to develop resistance to them.

CONCLUSIONS

The control of pathogenic microorganisms, which has been going on since the discovery of antibiotics, has led to the emergence of super pathogens capable of withstanding high concentrations of antibiotics and their combinations. It is said that it is possible to return to the pre-antibiotic era, when there were no means to combat infectious diseases; a future is possible, however, when there really will be no such means, because human activities will lead to the emergence of resistant forms of pathogens.

Obviously, the control strategy must be radically changed and focused not on killing the pathogens themselves but on influencing the factors of their pathogenicity and virulence. Numerous attempts to find such approaches can be seen in recent decades. They are generally targeted at inhibiting host-pathogen contact, cell adhesion, or immune system response regulation, which opens up the possibility of developing medications that can prevent disease progression even after the pathogen has entered the body. The development of new an-

timicrobials should be based on an in-depth study of the pathways and mechanisms of pathogen virulence, and therefore the choice of targets that are associated with non-vital cell processes and are least protected by pathogen cells. The development of such new generation drugs has the prospect of long-term effectiveness in overcoming the current problem of pathogen resistance and can provide a chance for the host organism with a low rate of evolution to successfully resist infectious diseases.

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ŠIUOLAIKINIŲ IR PERSPEKTYVIŲ ANTIMIKROBINIŲ MEDŽIAGŲ KŪRIMO KRYPTYS

Santrauka

Antibiotikų vartojimas vis labiau ribojamas dėl išsivystančio atsparumo patogeninėms bakterijoms. Laikui bėgant vis daugiau bakterijų taps atsparios antimikrobinėms medžiagoms, todėl būtina ištirti alternatyvius kovos su patogenais metodus. Tai padėtų sukurti pakankamai patikimą ir veiksmingą infekcinių ligų terapiją. Šioje apžvalgoje aptariami kai kurie naujausi gydymo įprastiniais antibiotikais ir neantibiotikais strategijų pokyčiai. Įrodyta, kad tradiciniai antibakteriniai taikiniai apima žinomų antibiotikų klasių darinius, naujas chemines klases su naujais taikiniai, taip pat nežinomus ar neapibrėžtus agentus. Buvo nustatytos daug žadančios kovos su mikrobų patogenais strategijos, įskaitant naujus taikinius, būtent – toksinų sekrecijos sistemas, bioplėvelės formavimąsi ir sukibimo mechanizmus, turinčius įtakos *Quorum Sensing* mikrobų populiacijoms. Be to, svarbu naujas antimikrobines medžiagas naudoti su kitais, ne antibiotiniais, veikimo mechanizmais – fagais ir fagų kilmės peptidais, mikrobiotą moduluojančias terapijas, stiprinančias imuninę atsaką.

Raktažodžiai: įprastiniai antibiotikų taikiniai, neantibiotikų taikiniai, gydymas neantibiotikais, infekcijos, pasipriešinimas