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Progress in resistance mechanisms to β-lactamase inhibitors

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Abstract. One of the main causes of death for people is bacterial infections. Among them, enzyme-mediated antibiotic resistance has been a serious problem and is a serious threat to public health security. The use of enzyme inhibitors can effectively deal with this problem, and antibiotics can be used in conjunction with inhibitors to partially restore their activity and enable them to function as antibacterial agents. Therefore, the development of new enzyme inhibitors is particularly important. In this paper, β-lactamases were analyzed and studied, and the resistance mechanisms of two different types of β-lactamases were reviewed. In addition, it is summarized that the enzyme inhibitors that can block the antibacterial effect of the enzyme and can reactivate the activity of the antibiotic. However, there is still a gap in the research of metallo-β-lactamases inhibitors, which is also the main direction of research and development in the future.

Keywords: Antibiotic resistance; hydrolase; enzyme inhibitors.

1. Introduction

Antibiotics are one of the greatest inventions of the 20th century. In the pre-antibiotic era, the vast majority of human deaths were attributed to infection. However, antibiotic resistance has been an unobserved epidemic. With the continuous emergence of antibiotic-resistant bacterial strains, which has seriously threatened human disease treatment and medical system, multi-drug resistant (MDR) bacteria spread widely in society, Humanity has entered the post-antibiotic era [1], and bacterial multi-drug resistance has become one of the most urgent health problems worldwide [2]. The failure of common antibiotics to treat these diseases has also made this problem more and more serious. Although billions of dollars and decades have been invested, the research progress of new antibiotics against these resistant bacteria is slow, the challenge is difficult, and more and more different methods are used to study new antibiotics.

So far, the decrease in permeability of cell membranes, the modification of surface targets' chemical structures, and the inactivation of antibiotics by drug-resistant enzymes are some of the molecular mechanisms of antibiotic resistance [3]. With excellent potential and research value, the most promising technique for fighting antibiotic-resistant bacteria is inhibition of bacterial resistance enzymes. [1]. The emergence of β-lactam antibiotics is an important time point in the fight against bacteria in humans. These highly effective and inexpensive products have been the mainstay of treatment against infections for the last century. However, the emergence of β-lactamases is gradually threatening the effectiveness of these drugs [4]. For example, carbapenems can be hydrolyzed by metallo-β-lactamases (MBLs) like New Delhi metallo-β-lactamase 1 (NDM-1) [5]. Therefore, it can provide effective means for us to fight

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against drug-resistant bacteria by studying the inhibitors of drug-resistant enzymes. Compared with conventional antibiotics, which directly inhibit the bacterial growth process or the required components, enzyme inhibitors have almost no components that directly cause bacterial death. However, these enzyme inhibitors have the ability to selectively bind to drug-resistant enzymes' active regions or impede the drug-resistant genes' transcription and translation processes, resulting in the decrease or even disappearance of drug-resistant enzyme activity [1]. One of the most obvious advantages is that drug-resistant enzymes do not have great selectivity for bacteria like traditional antibiotics, so they can delay the generation and evolution of drug-resistant bacteria as much as possible [6].

In this review, the discovery and research progress of β-lactamase inhibitors are mainly introduced. In this paper, the structure of β-lactamase inhibitors and the mechanism of enzyme inhibition are discussed. The development of enzyme inhibitors and their synergy with antibiotics can solve the problem of bacterial production of β-lactamase when antibiotics are used.

2. Antimicrobial mechanisms of β-lactam antibiotics

The β-lactam ring is present in all β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and β-lactamase inhibitors [7]. It has the ability to prevent the synthesis of peptidoglycan, which is the primary structural component of bacterial cell walls, particularly those of Gram-positive bacteria. Transpeptidases, which are significant peptidoglycan synthetases in bacteria and are in charge of polymerizing and cross-linking peptidoglycan glycan chains, represent the last stage of peptidoglycan synthesis. β-lactam antibiotics can irreversibly covalently bind to penicillin-binding proteins, thereby blocking the peptidoglycan biosynthesis process, leading to bacterial lysis and death [8].

3. β-lactamase-mediated resistance

The causes of bacterial resistance to β-lactams include: first, modifying or changing the target of antibiotics, which is used by Gram-positive bacteria to develop drug resistance; The second is to encode β-lactamase to hydrolyze antibiotics; The third is to reduce the extracellular permeability and limit the entry of antibiotics into the cells; The fourth is to improve the expression of drug efflux pump, which will expel the antibiotics that enter the cells. Of these, hydrolysis of antibiotics by enzymes is the most prevalent mechanism of resistance. This review will focus on β-lactamases.

β-lactamases are widely distributed in a variety of clinical pathogens and environmental microorganisms, and are one of the common hydrolases. The main types of β-lactamases that can be inactivated include penicillins, cephalosporins, monocyclic β-lactamases and carbapenems.

3.1. Classification

In 1989, Bush classified all endoβ-lactamases of bacterial origin into substrates, enzyme inhibitors, and molecular structures. On this basis, Bush proposed the latest classification of β-lactamases by synthesizing various methods in 1995, and formed the now widely accepted Bush-Jacoboy-Mederirios classification, referred to as Bush classification. According to the different substrate and inhibitor spectrum, he divided the enzyme into four types: the first type is cephalosporin enzyme, which is not inhibited by the commonly used β-lactamase inhibitor clavulanic acid. It is a class C in Almber's classification, mostly chromosome-mediated, and a small part of plasmid-mediated. The second type can be inhibited by β-lactamase inhibitors, mostly penicillinase, which can be divided into eight subgroups, including a-f, according to their activity against different antibiotics. There are many kinds and numbers of enzymes in this group, and most of them are plasmid-mediated. Subgroup 2b was further divided into 2be and 2br. Group 2be was extended-spectrum active, representing ESBL, and could inactivate the third-generation cephalosporins (cefotaxime, ceftazidime, cefixime). The 2br group indicates that the enzyme is not sensitive to clavulanic acid, and TEM-derived β-lactamase resistant to enzyme inhibitors is its alias. Of these eight subgroups, only the 2d subgroup belongs to class D in Almber's method, and all the remaining seven groups belong to class A. Group 3 is composed of three subgroups (a-c), corresponding to class B in Almber's method. Its activity depends on the participation of metal ions (mostly Zn2+), so it is also called metallo-β-lactamases, and is mostly mediated by

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chromosomes. This group of enzymes can inactivate almost all β-lactam antibiotics, including penicillin, cephalosporin and carbapenems. For example, NewDelhimetallo-β-lactamases-1(NDM-1), which attracted worldwide attention in 2010, is a highly resistant metallo-β-lactamase. It is a difficult problem to solve at present, and special attention should be paid to its identification in clinical practice. The fourth group is chromosomally mediated penicillinase which is more resistant to clavulanic acid, and there is no corresponding classification for this enzyme.

3.2. Mechanisms of β-lactamase-mediated resistance

Antibiotic hydrolysis can be inactivated by two primary forms of β-lactamases. One is serine-β-lactamases (SBLs) containing serine residues in the active site, which are divided into class A, C and D β-lactamases according to Ambler's classification. The other is metallo-β-lactamases (MBLs) whose activity is dependent on metal ions (mostly Zn2+), which are class β-lactamases according to Ambler's classification.

3.3. Serine -β lactamases

TEM enzymes, SHV enzyme, and AmpC enzyme have been the predominant SBLs in the mechanism of β-lactam resistance throughout the past few decades [9]. The main mechanism of their hydrolysis of antibiotics is that they bind to β-lactam antibiotics to form non-covalent Michaelis complex, which is cleaved and opened to form acylated products under the attack of serine nucleophiles. After hydrolysis, the active enzyme is obtained, leaving the inactive ring-opening product, and its catalytic mechanism has been well established. Currently, the main SBL inhibitors in the market are clavulanic acid, sulbactam and Vaborbactam.

A common β-lactamase inhibitor that comes from natural sources is clavulanic acid. It was first isolated from the fermentation broth of Streptomyces corynebacterium in 1976. Its antibacterial activity is very low on its own, but it has strong inhibitory activity against many clinical β-lactamases. By acting on the active site on the surface of the enzyme, clavulanic acid firmly binds to the enzyme, causes its inactivation, and plays the role of protective antibiotics. This phenomenon is stable and irreversible.

Sulbactam is a penicillin-sulfoxide, which is a synthetic irreversible competitive β-lactamase inhibitor first developed in 1978. Its structure is similar to penicillin. The antibacterial effect of sulbactam is weaker than that of clavulanic acid, but its stability is higher, and its IC50 to TEM-1 and SHV-4 is 1.56 μmo/L and 0.26 μmo/L, respectively [10]. Combined with benzicillin and piperacillin to form a compound preparation can improve the antibacterial spectrum and show synergistic effects. It is generally used in clinics by intravenous or intramuscular injection.

In 2017, vaborbactam, a new β-lactamase inhibitor based on boron, was made accessible. It comprises cyclic α-amylboronic acids and produces borate ester rings, which facilitate the establishment of a favorable conformation and improved function for the bound structure. The covalent structure formed by boric acid and serine hydrolase can have tetrahedral structures in acylated and deacylated states, and boric acid is a very safe compound. Vaborbactam combined with meropenem can effectively inhibit the activity of Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria and enhance the antibacterial effect against Gram-negative bacteria. Especially for carbapenem Enterobacteriaceae, the inhibition rate of meropenem-vaborbactam with an inhibitor concentration of 8 μg/ml on Klebsiella pneumoniae was 96.6%, and the inhibition rate of Escherichia coli and Enterobacter cloacae was 100% [11].

Currently available SBLs inhibitors can both successfully improve the resistance of β-lactam medications in clinical practice and reduce the development of bacterial resistance to β-lactam antibiotics when used in combination with β-lactam antibiotics. At present, there are a variety of new SBLs inhibitors under research and development. To deal with more complex situations in the future.

3.4. The metallo -β lactamases

Metal-β-lactamases are class B metal-proteins. Most of these compounds are multimers in chemical structure. Metal zinc ions are an important cofactor in the multimers. Finally, it cleaves the weak amide

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bond in the β-lactam structure, thereby inactivating β-lactam antibiotics. As the nucleophilic group of these enzymes, the hydroxyl group of zinc-binding compounds can promote the inactivation and hydrolysis of β-lactam antibiotics, including penicillins, cephalosporins and carbapenems, and even prevent the hydrolysis of certain β-lactamase inhibitors, like clavulanic acid and sulbactam. In addition, the SBLs inhibitors on the market do not inhibit metallo-β-lactamase, and there is no effective and legally permitted metallo-β-lactamase inhibitor in clinical practice, which cannot solve the problem of drug resistance caused by MBLs. Therefore, it is urgent to develop an inhibitor that can inhibit metallo-β-lactamase.

In 1966, the first MBL was detected in Bacillus cereus gram-positive bacteria. According to the amino acid sequence, substrate distribution and metal ion number, MBLs can be divided into three subclasses: B1, B2 and B3. Most of the current clinical isolates belong to the B1 subclass, which is also the main research direction at present, including New Delhi metalloβ-lactamase (NDM), Veroniminase (VIM) and iminopenolactone (IMP). In this article, some of the latest ideas and methods for the development of MBLs inhibitors are introduced.

The combination of cefepime +VNRX-5133 (tanibactam) is a novel β-lactamase inhibitor that inhibits many important MBLS, including NDM-1 and IMP-1, with a stronger inhibitory effect on NDM. Several studies have found that most NDM-producing Enterobacteriaceae can restore the antibacterial effect of cefepime at 4ug/ml of VNRX-5133. However, the antibacterial effect of cefepime +VNRX-5133(0.12-8 μg/ml+4μg/ml) against Enterobacteriaceae IMP was very weak. VNRX-5133 (4μg/ml) can produce synergistic effect with meropenem (0.016~1μg/ml) in addition to cefepime, and has antibacterial activity against MBL-positive Enterobacteriaceae [12]. Cefepime +VNRX-5133 is a very promising combination in clinical application.

Aztreonam was approved by the FDA in 1986 and is the only clinically available monocyclic lactam antibiotic targeting MBL because it has a very stable structure. Among them, the combination of SBL inhibitors with aztreonam is a promising treatment option, and regulators is currently reviewing aztreonam/avibactam in the clinical applications of collaborative treatment [13]. Ceftazidime/avibactam combined with aztreonam is a regimen recommended by the Infectious Diseases Society of America for the treatment of MBL producing Enterobacteriaceae, which has been used in clinical practice and has shown good results. Furthermore, certain novel BLIs, including avibactam and zidbactam, are now being developed for use in clinical settings in conjunction with various β-lactams. By combining these inhibitors with aztreonam, a new treatment option for the issue of resistance in MBL may be possible. Fifty-five sequenced MBLs-producing Enterobacteriaceae strains were obtained, including 41 strains producing VIM, 10 strains producing NDM and 4 strains producing IMP. They were resistant to three commonly used antibiotics, including imipenem, meropenem and ertapenem. These MBL-producing strains have an 18.2% aztreonam sensitivity rate. During the experiment, zidebactam and avibactam have a very good synergistic effect with aztreonam.

4. Conclusion

The combination of β-lactam antibiotics and β-lactamase inhibitors is one of the successful strategies to deal with the problem of antibiotic resistance, and this scheme can effectively avoid the further development of bacterial resistance. This review analyzes and discusses two β-lactamases that can hydrolyze and inactivate antibiotics. It is found that people have enough understanding of the resistance mechanism caused by serine-β-lactamases and countermeasures, and the corresponding clinical drugs have been developed, such as clavulanic acid and sulbactam. However, as the research of compounds against metallo-β-lactamases is still in its infancy, many drugs targeting SBL mostly inactivate MBL. In addition, there are very few researchers to study the mechanism of action of the inhibitors of the MBL, so this article only discusses the relevant MIC experimental data of new drugs, did not further discuss its mechanism of action, but this article is hoped that can inspire more researchers to further study the drug resistance mechanism, perfect the experimental data, For the MBL caused by bacterial infection to provide more choice and treatment, and can be applied to clinical as soon as possible.

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