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Covalent Inhibitors Targeting FabH: A Cutting-edge Strategy in the Development of Novel Antibiotics

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Abstract. Antibiotic resistance has become a global public health crisis, and there is an urgent need to develop new mechanism antibacterial drugs targeting specific targets of bacteria. β -ketoacyl-ACP synthase Ⅲ (FabH) inhibitors have shown great potential to overcome bacterial resistance and have become an emerging hot field in the research and development of anti-infective drugs. And unlike traditional reversible inhibitors (relying on transient non-covalent interactions), covalent inhibitors can form stable covalent bonds to achieve irreversible inhibition of the target. This combination method brings significant advantages (such as long-term effectiveness and high efficiency, etc.). This article systematically reviews the uniqueness of FabH, analyzes the chemical structure and application of representative covalent inhibitors of FabH, dissects the mechanism of action of covalent inhibitors and the clinical evidence that FabH can be used as a covalent inhibitory target. The research on covalent inhibitors based on FabH provides a promising new approach for the development of the next generation of novel antibiotics that are highly efficient, narrow-spectrum and less prone to drug resistance.

Keywords: Antibiotic resistance; Fatty acid synthesis; FabH; FabH inhibitor; Covalent inhibitor; Development of antibacterial drugs.

1. Introduction

In recent decades, due to the abuse of traditional antibiotics, a global drug crisis has emerged, characterized by the increasing drug resistance of "superbugs" and the inactivation of immunity to traditional drugs, seriously threatening human survival. In May 2014, the World Health Organization released a report titled "Antibiotic Resistance: A Global Surveillance Report". The report documents the situation of antibiotic resistance in all regions of the world, especially for antibiotics used as a "last resort". In some parts of Africa, as many as 80% of Staphylococcus aureus infections are resistant to methicillin-resistant (MRSA), more than half in the Eastern Mediterranean region, and as much as 60% in Europe. This all means that treatment with standard antibiotics is ineffective [1].

The issue of antimicrobial resistance (AMR) has currently become the most complex threat to global public health at present. According to statistics, in 2019, 1.27 million deaths worldwide were directly attributed to the AMR, and 4.95 million deaths were related to the AMR. The direct number of deaths caused by AMR exceeded that of AIDS and malaria (the deaths from the two diseases were 860,000 and 640,000 respectively). AMR has become one of the main causes of human death. The latest report of the United Nations shows that if left uncontrolled, and by 2050, the number of annual deaths due to AMR will increase to 10 million, causing an economic loss of nearly 100 trillion US dollars [2,3]. With the development of molecular biology techniques, scientists have identified many targets of antibacterial drugs and carried out relatively in-depth research. In recent years, the key enzymes involved in the bacterial fatty acid biosynthesis pathway have attracted extensive attention from researchers. FabH is not only the rate-limiting enzyme of the entire FAS-II pathway, but also the "goalkeeper" that determines the specificity of fatty acid chain initiation and extension. The high conservation of its catalytic active sites and relatively unique structural characteristics make it an ideal target for development of the new and selective antibacterial drugs. Covalent inhibitors form a more robust binding effect with the amino acid residues of target proteins through covalent bonds, thereby better exerting the inhibitory activity of target proteins. It binds to the target protein through

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irreversible or reversible covalent bonds, and has the advantages of long-lasting effect, strong efficacy and overcoming drug resistance mutations. It has attracted much attention in the design of FabH-targeted drugs.

Therefore, this paper intends to systematically review the structural and functional characteristics of FabH, deeply explore the research progress of FabH inhibitors and covalent inhibitors targeting FabH and analyze the challenges and future development directions faced in this field.

2. Fatty Acid synthesis (FAS)

FAS is a crucial process in cell physiology. Based on the different composition patterns of the active centers, FAS is divided into FAS I and FAS II. FAS I is composed of a single polypeptide chain and exists in mammals and yeast. FAS II is mainly distributed in bacteria and plants and is composed of multiple independent small proteins. Each step of the reaction is catalyzed by different single-function enzymes. Although they are structurally and functionally related, the overall sequence lacks homology. Mycobacterium tuberculosis is rather special and possesses both the two types simultaneously. FAS I is responsible for the de novo synthesis of medium-chain (16C-24C) fatty acids. These products then condensing with long-chain (48C-54C) acyl-ACP to form mycoacid, a key component of the cell wall. Type II FAS cannot be synthesized from scratch. Its function is to extend the products of type I FAS and generate longer-chain acyl-ACP [4].

3. FabH

β-ketoacyl-ACP synthetase (KAS) contains three subtypes: KAS I (FabB), KAS II (FabF), and KAS III (FabH). FabH is the enzyme that has been studied most thoroughly. The molecular mass of E.coli FabH is approximately 33.5 kD. It also exists in the form of a dimer in solution, and the catalytic pocket contains three key amino acids, namely Cysll2, His244, and Asn274 [5,6].

It can be seen from this that the uniqueness of FabH is reflected in multiple aspects: (1) As the initiating enzyme for the fatty acid biosynthesis, it catalyzes the first step reaction of the pathway; (2) Regulated by the feedback inhibition of the end product palmitoyl-ACP, it plays a key metabolic regulatory role by terminating the cycle; (3) The gene sequence and substrate specificity are distinct from other KAS enzymes, specifically utilizing acetyl-Coenzyme A (rather than the acyl-ACP required by FabB/FabF) as the substrate; (4) The sensitivity to the classic KAS inhibitors, leptomycin and thiolactomycin, was significantly lower than that of FabB/FabF. (5) Phylogenetic analysis showed that FabH was widely present in clinical pathogens such as the Gram-positive bacteria, the Gram-negative bacteria, the chlamydia, the anaerobic bacteria, the mycobacteria and protozoa, and was an essential protein for the survival. However, other synthetase genes such as FabA, FabB were not conserved in all pathogenic bacteria [7].

FabH is indispensable in the bacterial FAS pathway and plays a decisive role. FabH inhibitors selectively inhibit bacterial systems by suppressing the activity of FabH and have relatively low toxic and side effects. Therefore, they are expected to become new broad-spectrum antibacterial drugs

4. FabH Inhibitor

4.1. Cerulenin

Cerulenin is a natural metabolite of Cephalosporin blue, with the chemical name (2R,3S)-2, 3-epoxy-4, keto-7, 10-trans, trans-1, 2-carbodienoic acid amide. It can specifically form covalent binding with the cysteine thiol, the active site of B-ketoacyl synthase in fatty acid synthase (FAS), at the epoxy site to inhibit FAS activity. The possible signaling pathways are through the inhibition of the mitochondrial pathway and the inhibition of FKB The expression and activity of it promote the apoptosis of tumor cells and inhibit the proliferation of tumor cells.

Cerulenin significantly inhibits the proliferation, the migration, the invasion, and also the glycolysis in breast cancer cells. Bioinformatics analysis suggested PKM2 as a potential target of cerulenin. Notably, the ErbB2 signaling pathway was found to upregulate PKM2 protein expression [8].

In addition, animal experiments were conducted to establish a nude mouse tumor model of human osteosarcoma cell line U2-0S BALB/C. The results showed that the tumor volume inhibition rates in the high/low dose groups were 51.2% and 24.2%,and the tumor weight inhibition rates were 49.1% and 25.5%, respectively. The comparisons of tumor volume and tumor weight among each group showed statistically significant differences. In the control group, obvious ultrastructural changes of cell apoptosis occurred, with smaller tumor cell volume, disappearance of microvilli in most cells, and increased cytoplasmic density [9].

4.2. Platencin and Platensimycin

Platencin and Platensimycin are respectively potent broad-spectrum antibiotics derived from Spanish soil (Streptomyces platensis) and South African soil (Streptomyces sp.). The two have similar structures and lower toxicity. The absolute configuration Platensimycin was confirmed by X-ray single crystal diffraction and 2D NMR. The structure of platencin was resolved by 2D NMR. Based on its similarity to the crystal structure of platemycin, Singh et al. inferred that its absolute configuration should be consistent with that of platemycin - this is due to the similar biosynthetic pathways of the two. The discovery of these two compounds is of great significance. They were screened out by using innovative whole-cell high-throughput screening technology (targeting ribosome function), targeting the key new target of multi-drug resistant bacteria - fatty acid synzyme II (FASII), and have a brand-new molecular skeleton. Therefore, they are recognized as landmark achievements in modern antibiotic research.

The biological activity experiments indicated that platinamycin had broad-spectrum antibacterial activity. The IC50 value for saFabF was 48 nM, while for saFabH it was 67 μM, and the MIC value for S. auureus and S.pneumoniae was 0.5-1 μg/mL. And cytotoxicity was not manifested in the mouse model. Tablets are dual inhibitors of FabF (IC50 = 4.6 μmol·L-1, Staphylococcus aureus) and FabH (IC50 = 9.2 μmol·L-1, Staphylococcus aureus). Its MIC for common Gram-positive bacteria was 0.06-4 μg•mL-1 [10,11].

4.3. Indole Derivatives

Indole is a common precursor of drugs. In recent years, Glaxo Smith Kline Company in the United States has discovered a new type of FabH inhibitor through high-throughput screening, using carboxyl-containing indole compounds such as SB-418011 and Compd.1. This type of compound has a stronger inhibitory effect on FabH of Escherichia coli and Streptococcus pneumoniae than on cER and TLM, while has no inhibitory effect on FAs I and has good selectivity [12].

The structure-activity relationship study of the indole FabH inhibitors reveals that both the 2, 6-dichlorobyl and carboxylic acid groups are active essential groups. The former generally functions by forming structural complementarity with the hydrophobic region of the active site, while the latter forms the key ionic interactions with arginine residues in the pocket. On the premise of retaining these two groups, the indole ring mainly serves as a scaffold. Li Song's research group verified this structure-activity relationship and found that replacing 2, 6-dichlorobenzyl would lead to a significant reduction in activity. They further expanded the framework and successfully replaced the indole ring with another five-membered heterocyclic ring to develop a class of pyrrole derivatives with excellent activity. Some of these compounds had antibacterial activity against Mycobacterium grassinosa reaching or even surpassing that of the positive control [4].

4.4. Secnidazole and its Derivatives

Secnidazole is a new generation of 5-nitroimidazole antibacterial drugs. Structurally, secnidazole is largely similar to metronidazole and tinidazole, but the difference lies in the substituted cyclic nitro-

o-nitro group. Compared with traditional nidazole drugs, secnidazole drugs have the characteristics of strong activity, few side effects, short treatment cycle and good tolerance, and therefore have received extensive attention from researchers. Currently, they are mainly used in clinical practice to treat urethritis and vaginosis caused by Trichomonas vaginalis, amoebic disease, and Giardia lamiformis.

Zhang et al. synthesized a series of the cinnamic acid derivatives based on the secnidazole skeleton and determined their antibacterial activities. Among them, the MIC of the compound with the best antibacterial activity was 1.562.56μg/ml, and the ICso value against E.coli FabH was 2.5μM, showing good antibacterial activity [13].

4.5. Oxime Ether Derivatives

Oxime ether compounds have attracted much attention due to their significant biological activities, especially antibacterial activities. A variety of antibacterial and antifungal drugs containing oxime ether groups have been developed. Antibacterial activity tests on a series of synthesized oxime ether derivatives (the test strains included Enterobacter human, Pseudomonas aeruginosa, Pseudomonas fluorescens, Enterococcus faecalis, Staphylococcus aureus, and Bacillus subtilis) showed that the MIC values of the most active compound test strains were as low as 3.13-6.25 μg/mL. Based on the analysis of the binding model between the most active compound and FabH, its molecular structure can precisely occupy the hydrophobic channels of the binding sites. The results of molecular docking further revealed that there are three key interaction sites between it and the protein, all of which involve aromatic rings,which strongly indicates that aromatic groups play the crucial role in the inhibitory activity [14].

Kosmalski et al. designed and synthesized 26 oxime ether compounds containing heterocyclic, alicyclic or aromatic groups, and conducted toxicity tests on HeLa cancer cell lines. The test results revealed that oxime ethers containing thiazole and benzothiophene structures could reduce the survival rate of HeLa cells when the concentration was 100-250 μg/m³ [15].

5. Covalent Inhibitors Targeting FabH

Covalent inhibitors are a class of small molecule compounds that bind to the amino acid residues of target proteins through covalent bonds. Such small molecules usually carry specific reactive functional groups, such as the acrylamide, the β -lactam, the sulfonyl fluoride or ethylene oxide, etc., which can undergo chemical reactions with the specific amino acid residues (such as cysteine, serine) in the target protein. Its development history is long and can be traced back to the advent of aspirin (an analgesic and anti-inflammatory drug) in 1897. Another milestone was the application of penicillin antibiotics in World War II in the 1940s [16,17].

Covalent inhibitors typically have two structures: a seeker and a warhead. They complete covalent binding to the target protein through two related but discontinuous steps: 1) Small molecule ligands reversibly undergo non-covalent interactions with specific binding pockets of the target protein through the seeker, forming the intermediate E·I; 2) The electrophilic elastic heads of the small molecule ligands in the intermediate complex will interact covalently with the nucleophilic amino acid residues of the target protein through reactions such as addition, substitution, and oxidation, generating the covalent complex E-I. This step of the reaction is usually slower than the first step [18,19].

Furthermore, unlike non-covalent inhibitors, covalently bound drugs do not conform to the equilibrium kinetic model. Even if they are excreted from the body, their inhibitory effect persists. This means that the exposure to the drug system reduced and non-target binding decreased, thereby lowering potential toxicity. It is precisely these characteristics that make covalent inhibitors powerful "hammers" targeting the key target FabH.

A study found that covalent inhibitors based on the 1,3, 5-oxadiazine-2-one skeleton can efficiently inhibit methicillin-resistant Staphylococcus aureus (MRSA). The lead compound Oxa1/Oxa2 (MIC as low as 0.25 μg/mL) was identified through phenotypic screening, and the target FabH, a key enzyme for fatty acid synthesis, was locked by whole-genome sequencing of drug-resistant mutant strains. Mechanism studies have shown that the compound achieves inhibition by covalently modifying the active site of FabH with cysteine Cys112. Mass spectrometry and crystal structure (PDB: 6KVS) have confirmed that this modification is highly selective - it does not attack glutathione, TEV protease or homologous enzyme FabF, which is attributed to the unique microenvironment of the FabH active pocket. This study provides a "new target - new mechanism - new framework" strategy for overcoming drug resistance, opening up a new path for the development of anti-MRSA drugs [20].

6. Conclusion

FabH, as the initiating enzyme and rate-limiting enzyme of the bacterial fatty acid extension cycle, plays an indispensable role in the survival of various pathogenic bacteria, and there are conserved nucleophilic cysteine residues in its catalytic active pocket. This unique structural feature makes it an ideal target for covalent inhibitors development.

Some FabH inhibitors with the potential to develop into new antibacterial drugs have been discovered clinically. Although they are still in their infancy, the research is in its infancy.

It also indicates that breakthroughs in this field still face key challenges: the precise design of covalent warheads is the core problem. It is necessary to ensure the cysteine reactivity against the FabH target while minimizing non-specific reactions with other nucleophilic groups in the host protein (such as other cysteines or lysines) to control the potential risks of off-target toxicity and immunogenicity. With the development of modern structure-based rational drug design concepts and emerging disciplines and technologies such as chemical biology, as well as a deeper understanding of the structure and properties of target proteins, this problem is bound to be effectively solved in the near future

Looking to the future, continuous research should focus on: 1) Discovering and optimizing new covalent warheads with higher selectivity and reaction efficiency; 2) Deeply analyze the structure of the covalent complex of FabH- inhibitors to guide the design; 3) Comprehensively evaluate its in vivo efficacy, safety and the tendency of drug resistance development in more complex infection models. The successful development of a safe and efficient covalent inhibitor targeting FabH not only holds the promise of providing a "blockbuster" level new weapon for clinical practice to combat drug-resistant bacteria, but also significantly enriches the mechanism library of anti-infinfection drugs, offering crucial strategic support for humanity to ultimately win the protracted battle against drug-resistant bacteria. This path represents an important dawn for the research and development of innovative drugs in the post-antibiotic era.

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