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POTENTIATION THE ANTIMICROBIAL ACTIVITY OF CEPHALOSPORINS ANTIBIOTICS

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Abstract

β-lactams have the most powerful and often administered class of antimicrobials in the health care center because of their excellent tolerance and potency. They have been used in clinical settings since 1964; cephalosporins have created significant worldwide burdens that make the bacteria develop resistance. Bacterial resistance to antibiotics could be innate or acquired resistance and classified into four types according to mechanisms, target site modification, change in membrane permeability, forced efflux from the cytosol and antibiotic inactivation. Cephalosporin resistance has developed worldwide, creating a serious risk to its continued use so it's very important to develop strategies to restore and maximize the performance of this important class of antibiotics such as cephalosporin derivatives, β-lactamase inhibitors, prodrugs, acting with intracellular bacteria and the use of drug nano-carriers. Among the most productive strategies to increase the performance of β-lactams is to use nanocarriers such as nanoparticles, liposomes, and niosomes. Nanocarriers may improve β-lactamases activity in the long run by minimizing resistance to antimicrobials and assisting in delivering the medicine to the target sites. The particle's surfaces are then modified using a particular type of surface active agent to allow medication release control and could be linked to antibodies or other recognition components allow recognition of a specific cellular target.

Cephalosporins

With more than 60% of the global antibiotic market, β-lactams are the most often utilized kind of antibiotic[1]. The active four-membered ring, the source of pharmacological properties, is their main distinguishing property [2]. At the same time, this ring is the weak point of these compounds as a target for the β-lactamase enzymes. For more than forty years, cephalosporins have been the well-known antibiotics that have maintained their allure among medicinal chemists [3]. They are produced via fermentation and semi-synthesis and are considered highly effective agents for treating many pathogens[4]. They are bactericidal medicines that block bacteria from forming cell wall [5]. The cell wall is primarily made up of peptidoglycan that functions as a barrier between the contents of the bacteria and the extracellular environment[6]. Peptidoglycans are obtained by enzymes found in the cellular membrane, known as Penicillin-binding proteins (PBPs). Cephalosporins have been in clinical use since 1964 [7] and their widespread usage has established

serious global burdens for bacteria to evolve resistance[8]. Resistance is the microbe's natural reaction to survive and being killed by the therapeutic substance[9]. The introduction of cephalosporins in the late 1960s resulted in hopeful versions with enhanced antibacterial properties, and resistance also started developing. Extended-spectrum antibiotics like ceftazidime and cefotaxime were created due to the discovery of β-lactam resistance[10]. The next step was finding blockers for the β-lactamase by employing structurally related chemicals to an antibiotic that can irreversibly block the catalytic cycle of hydrolysis [11]. In the 1970s, olivanic acids and clavulanic acids were discovered. Although clavulanic acid is a poor antibiotic, it is a very effective inhibitor for gram-positive and gram-negative β-lactamases. Then, many β-lactam molecules have been synthesized and seem to block β-lactamase hydrolyses, such as Ceftazidime, cefotaxime, and cefepime [11]. With antibiotic/inhibitor combinations and bacteria's high reproduction rate and mutational frequency, it's not unusual that resistance to β-lactamase inhibitors has developed [12].

Genetics of Antibiotic Resistance

Bacterial resistance to antibiotics can be:

Innate, which is characteristic of a particular bacterium and depends on biology of a microorganism (*Escherichia coli*) has an innate resistance to vancomycin)

Acquired resistance occurs from:

1-Acquisition of exogenous genes by plasmid, transposons, intagrons and finally bacteriophages see figure 1.

2-Mutation of cellular genes [13]

3-A combination of these mechanisms[14-17].

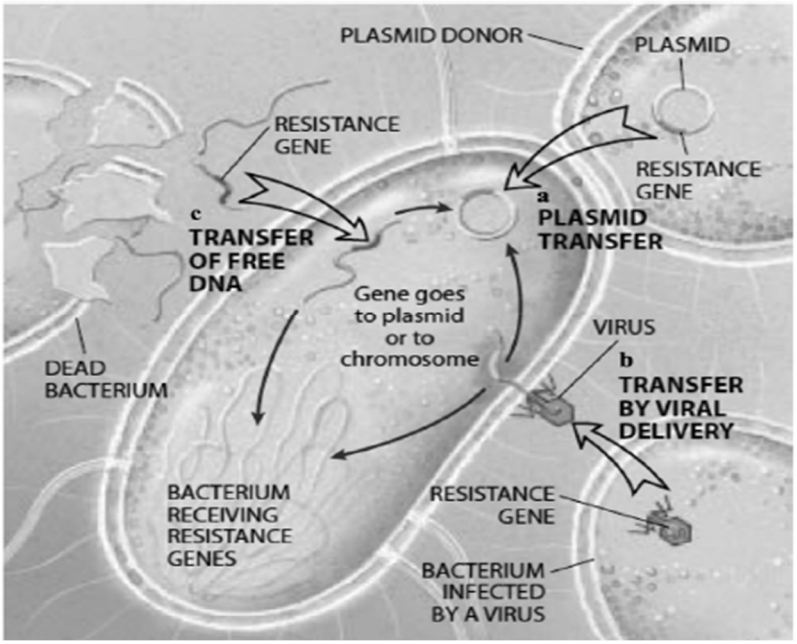

Figure 1: Three principal mechanisms of resistance gene transfer in a bacterium: (a) plasmid transfer, (b)transfer by viral delivery, (c)transfer of free DNA, cited from [13].

Types of Bacterial Resistance

Target Site Modification

PBPs, are the specific antibiotic target site[16]. PBPs mutation makes them less attractive to β-lactam antibiotics [18]. There are numerous examples of this kind of resistance, *Staphylococcus aureus* PBP2a, responsible for reducing MRSA sensitivity toward antibiotics [19]. The diminished susceptibility of PBP2a may be because of decreased interaction of these medicines to target spot and reduced later acylation [20].

A Change in Membrane Permeability

Causes antibiotic entry to a target protein to be more severely reduced. Porins, which are outer membrane protein water-filled channels that help small hydrophilic molecules cross this region, are the key regulators of drug penetration and susceptibility[21]. Cephalosporins are hydrophilic compounds that utilize these channels to get inside bacteria. Some gram-negative bacteria are notable for the ability to survive cephalosporins by altering their cell walls to yield an impenetrable barrier. Bacteria can restrict drug entry through compensatory or mutational changes into the porins to restrict the passage of antibiotics [22].

Forced Efflux from The Cytosol

This resistance is accomplished by the efflux pump, a crucial factor contributing to *Pseudomonas aeruginosa* and other gram-negative bacteria resistance [12]. Although in *Pseudomonas aeruginosa*, which has an extremely low permeability outer membrane, concentrations of several antimicrobials inside the bacterium are half of their external concentrations, indicating that external membrane alone cannot clearly describe the significance of resistance[23]. Recent research has revealed that several antibiotic efflux pumps are the second element in gram-negative bacteria's general intrinsic resistance see figure 2 [24].

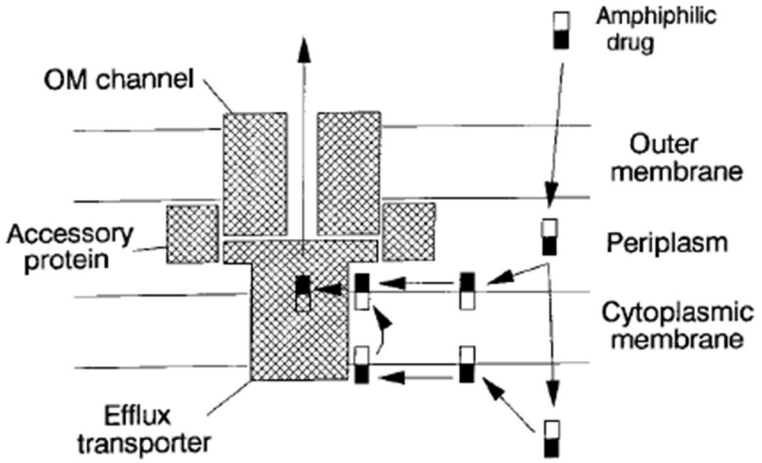

Figure 2: Hypothetical structure of efflux pumps, cited from [24].

Antibiotic Inactivation:

The principal source of resistance is a group of defensive enzymes known as β-lactamases, which can hydrolyze the four-membered ring and turn the antibiotic into an inert compound [25]. The serine- and metallo-lactamases are two separate groups of β-lactamases developed in bacteria. These families' various mechanisms explain why they behave in multiple ways when exposed to metal chelators, which significantly distinguishes the two enzymes by inhibiting metallo β-lactamases while not affecting the activity of serine β-lactamases[26]. Compared to metallo β-lactamase subcategories B2 and B3, the metallo β-lactamase genes for subclass B1 enzymes are more plasmid-borne and have greater therapeutic relevance[27]. This property means B1 metallo-β-lactamases can be relocated between bacterial types by the genetic elements. Aminoglycoside-modifying enzymes and chloramphenicol acetyltransferases are other enzymes involved in the inactivation process [28]. It's about 300 different *β*-lactamases. The most clinically important is produced by gram-negative bacteria [25]. *β*-Lactamases hydrolyze nearly all *β*-lactams that have ester and amide bonds, like penicillin; cephalosporin; monobactam; and carbapenem. This type of resistance occurs mainly by a new mutation on an already exciting enzyme so that it would be more active in the antibiotic destruction process[29]. Based on how closely their DNA sequences differ, β-lactamases are often grouped into four classes A; B; C; and D. To start bond hydrolysis, A, C, and D use a serine binding site. B enzymes are metallo lactamases that coordinate a nucleophilic hydroxide to facilitate ring opening using zinc ions in the active position [30-32].

Approaches to Control Bacterial Resistance:

The β-lactams have significantly impacted human health, handling a wide range of infections, from simple to life-threatening infections. Cephalosporin resistance has developed worldwide, creating a serious risk to its continued use. Therefore, there are potential for strategies to restore and maximize the performance of this vital group of antibiotics. Because of widespread use as an immense antibiotic family, cheap, and few adverse reactions, β-lactams are still beneficial for treating human bacterial infections. However, techniques must be proposed to maintain their antibacterial activity with minimum resistance [33, 34].

Powerful Cephalosporin Derivatives

More potent cephalosporin derivatives that have better efficacy toward β-lactamase hydrolysis also aggressive in targeting (PBPs) have in recent times been developed. This situation resulted in momentary success but ultimately lead to development of even more resistance. The revelation of cephalosporin C around mid-twentieth centuries paved the road for the creation of hundreds of novel cephalosporins [35, 36].

Class I

It was known that the cephalosporins used in medicine were losing efficacy when the TEM-1 penicillinase started appearing on plasmids in *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* [37, 38]. It was possible to hydrolyze early cephalosporins before 1980, rendering them unstable, which resulted in just a handful of their original molecules being retained. More potent

medications against Gram-negative infections have been discovered with the discovery of novel generations of these antibiotics [33].

Cephalosporin II

Cefuroxime was the only agent of this class to have both oral and systemic administration forms, but it was less stable to β-lactamase hydrolysis than class III or IV [39]. As seen with cefuroxime, cefpodoxime requires esterification with a proxetil group to achieve enough oral bioavailability for effectiveness [40, 41].

Cephalosporin III and IV

They were introduced into actual use in 1980 [39]. Cefotaxime, cefoperazone, and ceftazidime are categorized as subclass cephalosporin III, showing an expanded activity spectrum [39In the treatment of infectious diseases caused by gram-negative bacteria, these classes are crucial. Cefepime, is an expanded spectrum with enhanced resistance to familiar penicillinases SHV-1 and TEM-1 β-lactamase[42]. Because it penetrates the OmpF outer membrane porins more deeply, cefepime typically has reduced MICs against enteric bacteria. Ceftolozane and tazobactam have substantial antipseudomonal efficacy in a recently authorized combination for treating complex urinary tract and intraabdominal infections [43].

β-lactamase Inhibitors

The discovery of plasmid-borne TEM-lactamase in gonococci in the mid-twentieth century encouraged the pharma industry to focus on TEM-stable β-lactams and the expansion of inhibitors employed to achieve successful treatment. These results led finally to discovery of clavulanate and sulbactam [44]. This approach guides penicillin to the goal by preventing the damaging effect of β-lactamase. This strategy was initially successful but has subsequently met with opposition [27].

First Generation β-Lactamase Inhibitors

They are known as β-lactam-based suicide inhibitors (eg: clavulanic acid and sulbactam) that destroyed by β-lactamase but stay attached to the serine residue in the active site, therefore inactivating the enzyme [45].

Second Generation β-lactamase Inhibitors

Function as reversible inhibitors of extended-spectrum β-lactamases. Diazabicyclooctanones (class A carbapenemases and extended-spectrum β-lactamases, class C cephalosporinases, and some class D) are not based on β-lactams and include avibactam and relebactam. Unfortunately, none of these substances inactivate metallo β-lactamases in a clinically significant manner [45].

Difficulties in Design Metallo B-Lactamases Inhibitor

The commonly available serine β-lactamase inhibitors target the serine group that cleaves the β-lactam ring. This serine group is absent from metallo-lactamase active sites. Instead, zinc ions

excite a water molecule that pursues β-lactam hydrolysis. None of the presently available serine β-lactamase inhibitors are effective against metallo-lactamases. Moreover, the size and geometries of the two active sites are distinct. The considerable structural heterogeneity of metallo-lactamases, which consists of low similarity and variable Zinc content, poses an additional barrier to metallo-lactamases inhibition. In contrast to serine β-lactamases, which are largely microbial enzymes, metallo-lactamases belong to a family of metalloproteins with a variety of biological roles. In this family, around 30,000 genes are grouped [46].

Novel Strategies for Metallo-B-Lactamase Inhibitor Development

The zinc in metallo-β-lactamase stimulates the excitation of a water molecule, which breaks the -lactam ring. A reactive core intermediate with a negatively charged is created following this stage [46]. This intermediate interacts with the central metal ion to link to the active site. These intermediate organisms get a hydrogen from water before exiting the active site. In addition, this substance is missing from the serine-lactamase-catalyzed carbapenem hydrolysis process.

Understanding this manner has a major influence on design of metallo-lactamases inhibitors since it might inspire new strategies [47].

Novel Boronate Compounds

such as taniborbactam [48] and QPX772847 are effective against the majority of B1 metallo-β-lactamases. There is hope because both substances are undergoing phase 3 & 1 clinical studies, respectively [49, 50].

Metal Chelators

Which remove the necessary zinc ions, like Aspergillomarasmine A [51], or metal-based compounds that exchange the zinc ions for other metals can likewise render metalloxapase inactive. In the latter scenario, bismuth sub citrate [52] is involved. Because chelators resemble a natural defense process provoked by infectious diseases and involve substantial metal deposition by metal-binding proteins like calprotectin, their usage is attractive. Because chelators can also target numerous other metalloproteins, their low specificity is a significant issue when using them [53].

Tebipenem

The first orally administered carbapenem [54] is undergoing phase clinical trials. It is only marketed in Japan; therefore, This should be taken into account for future developments. Therefore, the development of oral β-lactamase inhibitors to be used in combination with this medication would be a huge step forward in the battle against antibiotic-resistant microorganisms [55].

Prodrugs

Dual-Action Prodrugs

The periplasm may contain hundreds of copies of this enzyme. As a result, a shift in approach is required for the death of such highly resistant β-lactamase-expressing bacteria. On their way to the PBPs, antibiotics and inhibitors must traverse the periplasmic area first. Concerns may develop if the two fragments do not simultaneously go into cell [56]. One suitable option is to utilize a dual-action drug that inhibits the targeted PBPs and the β-lactamase enzymes. Cephalosporin and β-lactamase inhibitors formed a compound by reversible pairing. This compound initially blocks the hydrolytic enzyme before dispensing the antibiotic [54].

Recently, multi-action drugs have been researched to release the antibiotic into the resistant bacterial cell. This method has the disadvantage of requiring the agents to be chemically synthesized, which is costly. The linking of the β-lactam antibiotic to the β-lactamase inhibitor in one molecule improves intracellular absorption and establishes that two substances reach their goals simultaneously. This compound could be represented as a prodrug, an inactive molecule that becomes active when it reaches its intended target spot. This method has numerous advantages of lesser breakdown and longer half-lives. We developed new antibacterial medications by linking clavulanic acid with either amoxicillin or cephalosporin 1-oxide. This progress was made using the dual targeting technique. Amoxicillin and clavulanic acid were connected via a butenolide bridge to produce a prodrug. Compared to their original medications, these conjugates demonstrated improved effectiveness against β-lactamase generating microbes and an improved permeation rate. Their significant values of MIC towards the β-lactases prove that β-lactamase inhibitor hydrolyzed them to release their amoxicillin [55].

Enzyme-Catalyzed Activation.

By attaching a cytotoxic moity with a β-lactam, the β-lactamase can effectively be exploited as a biosensor to create antibacterial drugs. This delivery strategy guarantees the active chemical is delivered to the target cell [9].

Delivery to Intracellular Bacteria

These arise when bacteria reside in human cells, which protects them from antibacterial medications [57]. Current findings on intracellular bacteria have revealed a remarkable recovery of β-lactams potency towards these microorganisms living inside host cells, raising optimism for their utility in treating latent bacteria that cause persistent illnesses. Ampicillin eradicates internal bacteria more quickly and thoroughly than their extracellular ones [58]. The discovery of MRSA and sensitive bacteria displayed equal meropenem susceptibility when phagocytosed into macrophages, this implied that MRSA susceptibility to β-lactams had been restored in the intracellular environment [59]. Antibiotic accumulation has been aided by the development and study of neutral charge β-lactam prodrugs. Pivampicillin is a prodrug that acts as an uncharged β-

lactam and deposited in cells [60], resulting in the formation of the active antibiotic ampicillin [9].

The Use of Drug Nano-Carriers

Among the most productive strategies to improve β-lactams functions is to use nanoparticles, liposomes, and Niosomes. Since it is interesting to deliver these hydrophilic antibiotics intracellularly, nanocarrier techniques are being researched to administer and avoid -lactamases. Nanocarriers may improve potency β-lactamases in long run by minimizing resistance to antimicrobials and assisting in delivering the medicine to the target sites. Utilizing these strategies helps to improve activity and to lessen drug resistance issues [61]. Microspheres and microcapsule loaded drugs were found in the 1970s then implanted for targeting infected organs [62]. Then, new dynamic and effective drug delivery vehicles were developed, including liposomes, nanospheres, and nanocapsules. The reticuloendothelial system removes endogenous plasma proteins from colloidal particles, preventing the liver and spleen's phagocytic cells from doing so. The particle's surfaces are then modified using a particular type of surface active agent to allow medication release control. Liposomes and nanoparticles linked to antibodies or other recognition components allow recognition of a specific cellular target [61].

Liposomes

With an aqueous core, the phospholipid bilayers made by the hydrophilic heads of the lipids. As a result, a water--soluble drug like penicillin may be held within the aqueous core by aligning hydrophobic tails. The lipophilic framework holds the lipophilic molecule [63]. Endocytosis is the process of release encapsulated medication from a liposome and enters the cell membrane. As a result, liposomes are perfect for delivering antibiotics [64].

Nanoparticles

Various treatments use nanoparticles as a drug carrier as well. Liposomes, on the other hand, have a tendency to break down more quickly. Nanoparticles, as a class, may facilitate effective drug administration because of their biocompatibility, biodegradability, consistent shape, and size. Enabling the antibiotic to remain active for an extended period of time, nanoparticles serve as drug reservoirs. Drug loading and ingredient release are influenced by the nanoparticles' components. 0.8 mg of ampicillin linked to nanoparticles as 96-mg dosages of free ampicillin worked for infected rats [65]. These nanoparticles also rise effectiveness of the β -lactam by 20 times see Figure 0-1 [12].

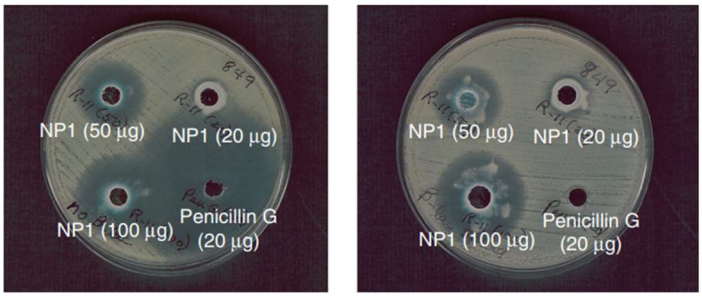

Figure 0-1: Penicillin Nanoparticles against S. aureus cited from [12].

When nanoparticles accumulate inside the liver and spleen, they are thought to be most efficacious. Hydrophilic antibiotic absorption is also enhanced by this method. However, no matter how much medicine is administered, there is no difference in the particle diameter. Greater molecular weight and more lipophilic surfactants release ampicillin more quickly [12].

Niosomes

Another example of a nanocarrier that in the field of antibacterial activity enhancement is niosomes. They can acquire the desired form, size, and membrane properties by altering their content. Studies are underway to improve antibacterial activity and decrease resistance [66]. Norfloxacin niosomes, which is fluoroquinolone antibiotics were examined on Pseudomonas aeruginosa[67]. When compared to medication solution, niosomes improve the efficacy, which is evaluated by the minimum inhibitory concentration and suppressed biofilm formation. Adding a positively charged substance to fluid niosomes significantly increased antibacterial activity [66].

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