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Cefiderocol, the first catechol-cephalosporin

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Mechanism of action of cefiderocol

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ABSTRACT

Gram-negative bacilli are intrinsically resistant to many antibiotics due to the low permeability of their outer membrane. The most effective strategy to solve this problem has been the design of antibiotics that cross the membrane using specific transport systems. This is the case of cefiderocol, which, unlike cefepime or ceftazidime, has a chlorocatechol group at the end of the C-3 side chain. This group is recognized by transporters located in the outer membrane that allow cefiderocol to accumulate in the periplasmic space. Furthermore, cefiderocol is not a substrate for efflux pumps and the configuration of the side chains at C-7 and in particular at C-3 confer it a high stability against hydrolysis by most beta-lactamases of clinical interest including class A (KPC, BLEEs), C (ampC) or D (OXA-48) serine beta-lactamases and metallo-betalactamases (NDM, VIM. IMP). In order to better understand the mechanism of action of cefiderocol, the importance of iron in bacterial metabolism and the competition for iron between bacteria and host are reviewed.

Keywords: cefiderocol, mechanism of action, Gram-negative, siderophore

INTRODUCTION

Gram-negative bacilli are intrinsically resistant to many antibiotics due to the low permeability of their outer membrane that slows the passive diffusion of hydrophobic compounds of high molecular weight, which are active against Gram-positive microorganisms. The outer membrane is an asymmetric lipid bilayer formed, in its superficial layer, by lipopolysaccharides (LPS) and, in its deeper or inner layer, by phospholipids of similar composition to those of the cytoplasmic membrane. The outer layer is less fluid (more rigid) than the inner layer because, unlike the phospholipid molecules that can move freely through the membrane, the negatively charged lipopolysaccharide molecules are neutralized and held together by divalent cations such as Mg2+. The outer membrane also contains two main classes of proteins, the lipoproteins and the proteins known as OMP (outer membrane proteins). The former bind the inner layer of phospholipids to the peptidoglycan and the OMPs can form channels (pores) that allow the passage of small hydrophilic molecules into the periplasmic space.

In addition to the difficulties of diffusion through the outer membrane, there are active transporters (ejector pumps) that can extract antibiotics from the periplasmic space or from the bacterial cytoplasm (Acrb-TolC in E. coli or MexAB-OprM in *Pseudomonas aeruginosa*). In *Enterobacteriaceae*, the structure of the most frequent porins (OmpF, OmpC) allows access to molecules with a molecular weight of 600-700 Da. On the other hand, the most frequent porins in *P. aeruginosa* (OprF, OprD), *B. cepacia* (OpcP1/OpcP2) or *Acinetobacter baumannii* (OmpA-AB), have a significantly lower permeability than that observed in *Escherichia coli*, which prevents the passage of molecules weighing more than 200 Da (the size of a monosaccharide) and are therefore called "slow" porins. This reduced permeability is compensated by: i) the secretion of nutrient-degrading enzymes, ii) the expression of a high number of nutrient-specific porins (OprB for glucose or OprD for basic amino acids) and iii) the expression of specific transporter proteins [1]. OprD is the main channel for entry of carbapenems through the outer membrane, and reduced expression or loss of OprD is frequently observed in carbapenem-resistant clinical isolates.

The slow penetration of the β-lactams through the porins, together with the removal from the periplasmic space by efflux pumps, allows the trapping and hydrolysis of the antibiotic molecules by the β-lactamases, before they reach the PBPs.

Different strategies aimed at increasing the concentration

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of antibiotic in the periplasmic space include i) the use of outer membrane sensitizing compounds, ii) the use of inhibitors of efflux pump activity, iii) modification of the structure or electrical charge of the antibiotic, and iv) the design of antibiotics that cross the membrane using specific transport systems such as siderophore-Fe3+ complex receptors.

To date, several outer membrane sensitizers have been studied. None of these sensitizers, with the exception of SPR206, has shown promising antipseudomonal activity. SPR206 is active against *P. aeruginosa* with potency similar to polymyxin B and is currently in clinical trial [2].

Inhibitors of efflux pumps include Phe-Arg-b-naphthylamide, D13-9001, polyamines and bacteriophage OMKO1. Phe-Arg-b-naphthylamide (PAbN) is a broad-spectrum peptidomimetic compound capable of interfering with the four clinically relevant RND (resistance nodulation division) efflux pumps of *P. aeruginosa*. It probably functions as a substrate for these pumps. However, both PAbN and derivatives of this compound have not been approved, as toxic effects have been reported during Phase 1 clinical trials. The pyridopyrimidine derivative D13-9001 is active against the MexAB-OprM efflux pump [3]. Specificity against a single pump limits its use to coadministration with antibiotics cleared exclusively by it. Polyamines are aliphatic carbon chains containing several amino groups. A polyamine structure has been identified as a strong efflux pump inhibitor without direct antimicrobial activity. Association with aztreonam, chloramphenicol or a tetracycline decreased the MIC_{90} by 5- to 8-fold [4]. Finally, the lytic bacteriophage (of the family *Myoviridae*), OMKO1 ("Outer membrance porin OprM Knockout dependent phage 1"), utilizes OprM of the MexAB and MexXY efflux systems as a binding site. Selection to resist attack by bacteriophage OMKO1 creates an evolutionary compensation in *P. aeruginosa* consisting of reduced OprM expression, leading to increased susceptibility to ciprofloxacin, tetracycline, ceftazidime, and erythromycin [5].

The passage of an antibiotic through a porin occurs by facilitated diffusion. The protein that forms the porin has a loop towards the middle of the channel that decreases its span. The amino acids in this region have electrical charges that cause an electrostatic interaction between the antibiotic or substrate and the channel wall. This explains the specificity of a given porin for a particular antibiotic, as is the case with OprD and carbapenems, whose structure and electrical organization are very similar to the natural substrate of the porin (basic amino acids such as arginine). Selective modification of an electrical charge in meropenem has shown improved passage through an alternative porin to OprM. However, this change reduces its ability to acylate with PBP and thus its antibacterial activity, although it retains some efficacy against *P. aeruginosa* OprD- strains [6]. In the future, the design of new antibiotics could incorporate the analysis of passage through one or more porins.

The most effective strategy to solve the problem of diffusion through the external wall has so far been the design of antibiotics that cross the membrane using specific transport systems such as the receptors of the siderophore-Fe3+ complex. This is the case of cefiderocol and to better understand its mechanism of action we will review the importance of iron in bacterial metabolism and the competition for it between bacteria and host.

RELEVANCE OF IRON IN BACTERIAL METABOLISM AND THE BATTLE FOR ITS ACQUISITION WITH THE HOST'S PROTEINS

Iron is an essential element for aerobic respiration. The respiratory chain in bacteria is located on the cytoplasmic side of the inner membrane and is composed of a set of proteins (complexes) that aim to create an electrochemical proton gradient by transporting electrons between molecules capable of donating and/or accepting 1 or 2 electrons (oxide-reduction reactions) to a final acceptor (oxygen) that allows the synthesis of ATP. The ferrous ion (Fe2+) is a good electron donor and therefore an essential element of the respiratory chain of eukaryotic and prokaryotic cells, which is transported through the chain attached to a cytochrome.

The intracellular concentration of iron necessary to guarantee the viability of a bacterium is 10-6M, this is a very high concentration if we take into account that the concentration of free iron in serum or in any tissue of the host is of the order of 10-24M. Under physiological conditions iron is bound to hemoglobin, in intracellular deposits such as ferritin or to extracellular proteins such as transferrin. At physiological pH transferrin has a high affinity for Fe3+, as pH decreases the affinity decreases and ferric ions are released into the medium. This circumstance occurs in a septic focus where the presence of organic acids reduces the pH. To counteract this situation and prevent bacteria from obtaining free iron, neutrophils synthesize lactoferrin, which has an affinity for Fe3+ 300 times higher than transferrin, and this affinity increases in an acidic medium [7].

HOW DOES A GRAM-NEGATIVE BACILLUS ACQUIRE IRON FROM THE INVADED TISSUE?

To reach the intracellular iron concentration necessary for their metabolism, bacteria synthesize molecules capable of binding iron with high affinity (association constants of 1020 to 1030 M-1) known as siderophores (e.g. enterobactin, pioverdin, salmochelins). These molecules are dumped into the medium to bind scarce free iron (Fe3+) for which they compete efficiently with host proteins [8]. The siderophore-Fe3+ complex is recognized by receptors located on the outer membrane that are able to move it to the periplasmic space with the help of protein complexes of the cytoplasmic membrane (TonB and ExbB/ExbD family proteins) that generate the energy necessary for active transport. Once inside the bacterium, the iron will be incorporated into the respiratory chain.

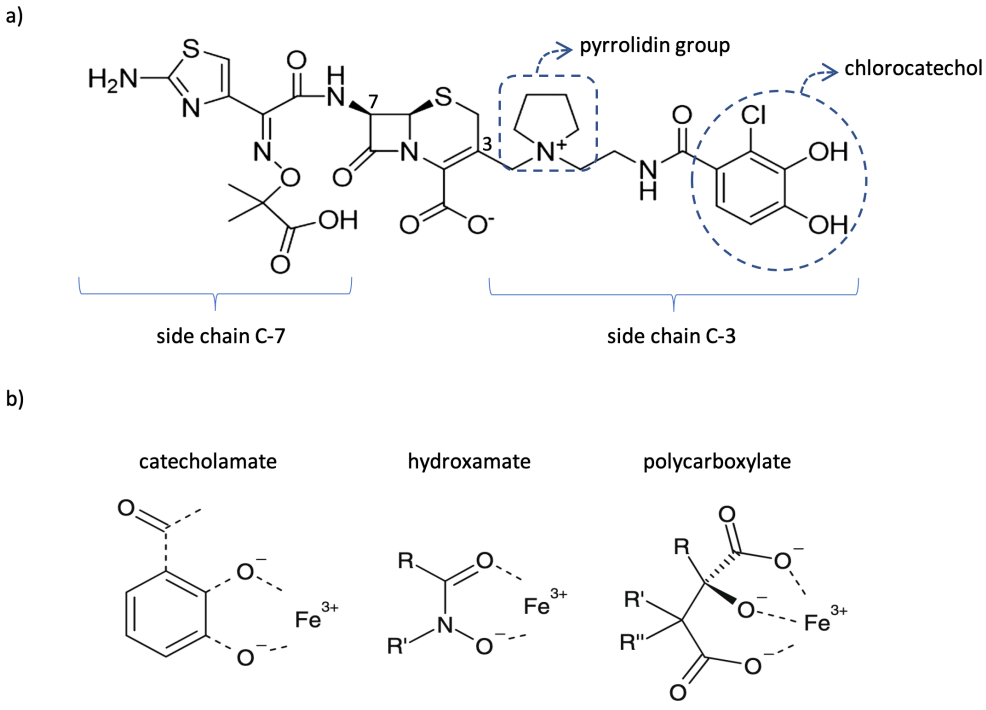

Figure 1 | **Molecular structure of cefiderocol (a). Structure of the group that fixes iron in different siderophores (b).**

MECHANISM OF ACTION OF CEFIDEROCOL

Cefiderocol is a cephalosporin with high affinity mainly for PBP 3 and a structure similar to that of cefepime due to the presence of a pyrrolidin group in the C-3 side chain, which confers potent antibacterial activity and greater stability against beta-lactamases. In addition, it possesses a carboxypropanoxymino group in the C-7 side chain similar to that of ceftazidime which improves transport across the outer membrane [9]. But unlike cefepime or ceftazidime, cefiderocol possesses a chlorocatechol group at the end of the C-3 side chain (Figure 1a) that confers siderophore activity. Siderophores can be grouped according to the structure fixing the iron atom into catecholamates, hydroxamates or polycarboxylates (Figure 1b). This catechol group is recognized by transporters such as CirA and Fiu in *E. coli* or PiuA in *P. aeruginosa* and allow cefiderocol to accumulate in the periplasmic space ("Trojan horse") avoiding resistance mechanisms such as loss of porins, Furthermore, cefiderocol is not a substrate for efflux pumps and the configuration of the side chains at C-7 and in particular at C-3 confers low affinity and/or high stability against hydrolysis of most beta-lactamases of clinical interest including class A (KPC, ESBLs), C (ampC) or D (OXA-48) serine beta-lactamases and metallo-betalactamases (NDM, VIM, IMP) [10].

CONFLICT OF INTEREST

AS has participated in scientific meetings and lectures organized or promoted by the companies Pfizer, MSD, Angelini, Shionogi, and Gilead.

JM has participated in scientific meetings and lectures organized or promoted by the companies Pfizer, and Shionogi.

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