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Drugs of the Future: Review

Cellular and molecular aspects of drugs of the future: meropenem

P. Cottagnoud

Department of Internal Medicine, Inselspital Bern, Bern (Switzerland), Fax + 41 31 632 38 47, e-mail: pcottagn@insel.ch

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Abstract. Meropenem, first synthesized in the late eighties, has become one of the most important β-lactam antibiotics of the carbapenem subclass used for the treatment of a variety of life-threatening infections. Due to its unique chemical structure, meropenem is not inactivated by the kidney dehydropeptidase I and the majority of microbial beta-lactamases. Its antimicrobial activity is based on its high affinity for the majority of cell wall-synthesizing enzymes, the so-called penicillin-binding proteins, of Gram-positive and -negative bacteria. However, bacteria have evolved several approaches to resist meropenem: (i) by reducing the affinity of the penicillin-binding proteins for the antibiotics, (ii) by decreasing the permeability of the outer membrane of Gram-negative bacteria, (iii) by using efflux pumps, and (iv) by activating zinc-dependent carbapenemases. Meropenem has a low toxicity profile and, in contrast to imipenem, no central nervous system toxicity.

Key words. Meropenem; antimicrobial action; mechanisms of resistance.

Historical overview

A major landmark in β-lactam research was the discovery of a new molecular family based on the carbapenen-2-em-3-carboxylic acid nucleus (see fig. 1) and represented by the olivanic acids and thienamycins [1, 2]. Their structures differ from the classical β-lactam antibiotics in that they harbor a highly strained 4,5-bicyclic ring system based on an unsaturated five-membered ring in which a methylene replaces the sulfur atom in position 1 and the C-6 acylamino-substituent of the bicyclic β-lactam ring is replaced by a carbon substituent, which is invariably an α-hydroxyethyl group [3].

During the past decades, scientists focused their efforts on discovering naturally occurring inhibitors of bacterial β-lactamases in order to overcome a major mechanism of resistance of microorganisms. Among the first β-lactamase inhibitors discovered were the olivanic acids, so called because they were isolated from a strain of *Streptomyces olivaceus* [4]. These new compounds were not only β-lactamase inhibitors but showed potent antibacterial activity. During that time, researchers at Merck managed to isolate the thienamycin family of carbapenems from *S. cattleya* [2]. Since then, a broad series of carbapenem analogues, e. g., carpetimycin A and asparenomycin A, have been isolated from *Streptomyces* spp. These new substances are powerful β-lactamase inhibitors with broad antibacterial activity.

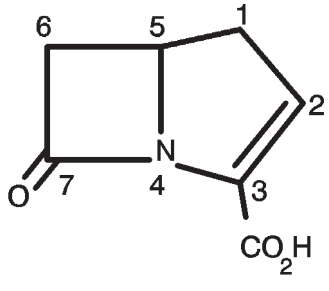

Figure 1. The chemical structure common to all carbapenems: a β-lactam ring fused with a carbapenem nucleus.

Following the first successful total synthesis of thienamycin, scientists realized that this new carbapenem, exhibiting exceptional antibacterial potency even against *Pseudomonas,* was concentration-dependent unstable. They found that the N-formimidoyl derivative (imipenem) was fivefold more stable than thienamycin in aqueous solutions [5].

Unfortunately, the first parenteral administration in animals and humans revealed that imipenem was rapidly degraded by a dehydropeptidase enzyme (DHP-1), located in the brush border of the kidney [6]. This unexpected complication was bypassed by the simultaneous administration of a potent, competitive inhibitor of the DHP-1 enzyme, namely cilastatin, in a 1:1 ratio with imipenem. In addition, cilastatin drastically reduced the nephrotoxic side effect of imipenem [7, 8].

Subsequently, pharmaceutical companies focused their research on the development of a single stable compound containing the antimicrobial potential of imipenem. By structural modification of the substituents of the carbapenem ring, the intense research of several companies led to the development of new carbapenems, e.g., panipenem [9] and, most particularly, to the synthesis of meropenem [10]. Meropenem (–)-(4R,5S,6S)-3-[[(3S,5S)-5-(dimethylcarbamoyl)-3-pyrrolidinyl]thio]-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-1-azabicyclo-[3,2,0]hept-2-ene-2-carboxylic acid; fig. 2] has an extremely broad antibacterial spectrum and a sufficient stability to DHP-1 to be administrated as monotherapy. The essential properties of meropenem, based on its unique chemical structure, are summarized in figure 2.

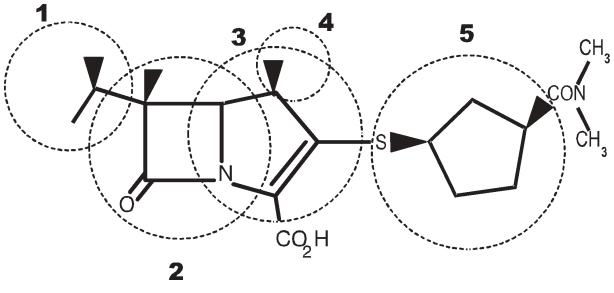

Figure 2. Meropenem. 1, the hydroxymethyl side-chain in trans configuration protects the β-lactam ring and affords stability against β-lactamases; 2, the β-lactam ring confers a high affinity for penicillin-binding proteins, target structure of all β-lactam antibiotics; 3, the carbapenem nucleus is responsible for an ultra-broad spectrum of antibacterial activity; 4, the methyl group at C1 provides considerably more resistance to renal DHP-1 than imipenem; 5, the C2 substituent is responsible for its high activity against *Pseudomonas aeruginosa* and other Gram-negative bacteria and in addition may account for the reduced proconvulsant activity.

Mode of action of meropenem: interaction with cell wall synthesis

As is the case with all β-lactam antibiotics, the antibacterial action of meropenem is based on inhibition of the last steps in cell wall synthesis. The rigid structure of the cell wall outside the plasma membrane is common to all bacteria and mainly responsible for their integrity. The major component of the cell wall is a framework of peptidoglycan, constructed according to a similar pattern in all bacteria. Linear glycan chains of alternative β1,4-linked N-acetylglucosamine and N-acetylmuramic acid are substituted through the D-lactyl group of N-acetylmuramic acid by a tetrapeptide side chain [11]. In *Escherichia coli,* this tetrapeptide consists of L-Ala-γ-D-Glu-L-A2pm-D-Ala (A2pm = meso-diaminopimelic acid) [12]. In Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus pneumoniae*, diaminopimelic acid is replaced by L-lysine [13]. Peptide side chains are linked together by means of bridges from the C-terminal D-alanine of one peptide to the ω-amino group of the diamino residue of another peptide. This unique linkage path leads to a variety of possible cross-links between the peptide side chains, conferring a highly complex three-dimensional structure to the bacterial cell wall. Over the past years, HPLC analysis of the peptidic part of the bacterial cell wall has provided new insights into the cross-linking mode of the cell wall. In pneumococci, among the best studied bacteria, the most frequent directly cross-linked dimer is a tritetrapeptide. A striking feature of the pneumococcal peptide network is the presence of both directly and indirectly cross-linked components. In the latter, alanyl-serine or alanyl-alanine dipeptides form the cross-link. The most abundant uncross-linked monomer of the peptidoglycan is a tripeptide (L-Ala-D-Glu-L-Lys). Based on the cross-linking mode, the pneumococcal cell wall may be classified as either A1α or A3α [14].

Synthesis of the cell wall is a process of multiple enzymatic steps. In brief, precursors (disaccharide peptides) are transported through the plasma membrane and incorporated into the nascent cell wall. The final step is the building of a peptide bound between the not yet cross-linked glycan chain and the pre-existing peptidoglycan. This reaction is carried out by transpeptidases. This transpeptidation leads to a three-dimensional network and confers its definitive structural rigidity to the cell wall. In *E. coli,* only 20–30% of the peptidoglycans are cross-linked, the remaining peptides are all tetrapeptides. The fifth amino acid (D-alanine) is removed by an enzyme called D,D-carboxypeptidase.

All enzymes involved in the last steps of cell wall synthesis, e.g., transpeptidase and carboxypeptidase, are anchored in the plasma membrane. They are also called penicillin-binding proteins (PBPs) because they are covalently attached and inactivated by β-lactam antibiotics. In *E. coli*, one of the most studied microorganisms, seven

PBPs are usually detected in the cytoplasmic membrane, with molecular weights ranging between 91,000–40,000 Da (PBP 1A, PBP 1B, PBP2-6) and two additional PBPs with a lower molecular weight (PBP 7: 32,000; PBP 8: 29,000).

S. pneumoniae possesses six PBPs with similar molecular weight and function. The high-molecular PBPs are BP 1A, 1B, 2A, and 2X. PBP 2B and PBP 3 are the low-molecular-weight PBPs [15–18].

In *E. coli*, the high-molecular-weight PBPs (1A, 1B, 2, and 3) function as transpeptidases and are essential for cell viability. They are therefore called 'essential PBPs'. PBPs 1A and 1B seem to play a key role in the extension of the cell wall during cell growth. PBP 2 controls cell shape and PBP 3 cell division. The most abundant PBPs in *E. coli* are the low-molecular PBPs 4, 5, and 6, which have a D,D-carboxypeptidase activity and probably control the degree of cross-linking of the cell wall. They are not believed to be essential.

The basic mode of action of meropenem does not differ from the other β-lactam antibiotics and is based on inactivation of these enzymes (PBPs) fulfilling multiple tasks in the last steps of cell wall synthesis. Thanks to a structural analogy with the natural substrate of these enzymes (the cell wall precursors), meropenem is able to bind these proteins covalently by acylating a serine hydroxyl group of the PBPs. The kinetics of the interaction between the β-lactam ring and PBPs is summarized in figure 3.

The first step is the formation of a non-covalent Michaelis complex between antibiotic and cell wall enzyme, followed by the formation of an acylated enzyme. The resulting complex is very stable and poorly available for external nucleophile attack (H_2O or R-NH_2). The antibacterial effect of this family of antibiotics relies on this crucial step. Thereafter, these enzymes are prevented from fulfilling their normal function in the wall synthesis.

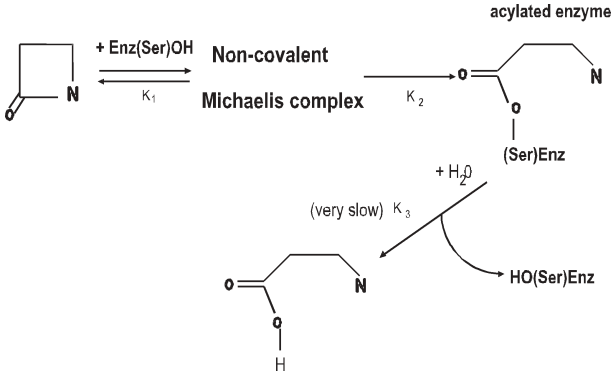

Figure 3. The essential biochemical steps of the interaction between the β-lactam ring and a serine residue of a PBP. The crucial reaction is the formation of a very stable acylated enzyme, preventing the enzyme from fulfilling its function in the synthesis of the cell wall [adapted from ref. 44].

This causes cell wall synthesis to halt, leading finally to the death of the microorganism.

Meropenem possesses variable affinities for the specific PBPs of different bacteria. Against *S. aureus,* meropenem has a high affinity for PBPs 1, 2, and 4, and low affinity for PBP 3. In *E. coli,* it exhibits the greatest affinity for PBP 2 but also binds effectively to PBPs 1A, 1B, and 3. Meropenem has a higher affinity for PBP 2 and PBP 3 than does imipenem [19, 20]. Although the interaction between PBPs and meropenem is well documented in the literature, the final mechanisms leading to bacterial cell death are far from being completely understood.

In addition, the antimicrobial effect of meropenem is enhanced due to its pronounced post-antibiotic effect (PAE) against a variety of Gram-positive and Gram-negative bacteria [21]. The PAE is the delay before microorganisms recover and start to regrow after a defined treatment period [22].

Mechanisms of resistance

Resistance against meropenem is based on four different mechanisms: (i) structural modifications of the target enzymes (PBPs); (ii) reduction of permeability through the outer membrane; (iii) the presence of β-lactamases, enzymes hydrolyzing the antibiotic before it reaches the target, and (iv) efflux pumps reducing the intracellular concentration of the drugs. These mechanisms are briefly discussed below.

Modification of the target enzymes

PBPs with a reduced affinity for carbapenems are a major cause of resistance in Gram-positive bacteria. This mechanism is common to all β-lactam antibiotics. In pneumococci, especially, PBP modification is based on a multiple-step process. In a first step, point mutations in the PBP genes lead to minor structural changes. In a further step, foreign DNA is imported, mostly from streptococci, and so-called 'mosaic PBPs' are built. These structurally modified PBPs have a drastically reduced affinity for all β-lactam antibiotics. This latter mechanism is responsible for the high-level resistance, concerning not only meropenem but all β-lactam antibiotics [23–25].

Another resistance mechanism has been developed by enterococci based on a 'by-pass mechanism'. *Enterococcus faecium* is able to adapt its enzymatic machinery to the presence of meropenem. PBP 3 as a cross-linking cell wall enzyme, which is susceptible to carbapenem, is inactivated by meropenem, but its function is taken on by PBP 5, which still acts as a transpeptidase and has a low affinity for carbapenems [26–27].

Methicillin-resistant staphylococci have evolved a different by-pass mechanism. Their resistance depends on the production of an additional PBP, called PBP 2′ or 2A, with transpeptidase activity and low sensitivity to all β-lactam antibiotics. The new PBP allows the bacterium to continue cross-linking of the cell wall when the other essential PBPs usually fulfilling this function have been inactivated [28–29].

Change of permeability

Change in the permeability of the outer membrane plays an important role in the resistance mechanisms of Gram-negative microorganisms, especially *Pseudomonas aeruginosa*. It is caused by loss of a porin-forming (D2) protein in the outer membrane. This porin forms small channels which are usually responsible for the uptake of basic amino acids, but also allow penetration of carbapenems [30–31]. These narrow channels are specific for the transport of carbapenems because D2-deficient mutants are still sensitive to cephalosporins and monobactams. The decrease in permeability alone is not always sufficient but needs to be potentiated by the presence of β-lactamases in *P. aeruginosa*, hydrolyzing the antibiotics which have reached the periplasm. [32]. In this regard, meropenem differs from other carbapenems, being unaffected by the chromosomal β-lactamases in *P. aeruginosa*, probably acting as a transient inactivator of β-lactamases [33].

Efflux pumps

Another mechanism preventing drugs from reaching their targets are the membrane-associated, energy-driven efflux pumps. These efflux pumps, conferring so called 'intrinsic resistance', play a major role in *P. aeruginosa*. The MEXAB-OprM efflux pump extrudes β-lactam antibiotics from the periplasm [34]. Meropenem is more affected by this resistance mechanism than other carbapenems. Mutants overexpressing this efflux system are more resistant to meropenem, but not to imipenem, than the wild type [35]. Meropenem seems to be a substrate for this pump because of its hydrophobic side chain at position 2, whereas imipenem and panipenem, with their strongly charged, hydrophilic side chains, cannot function as a substrate for this pump [36].

Zinc-dependent carbapenemases

As mentioned above, meropenem is usually resistant to *P. aeruginosa* β-lactamases, which have only weak carbapenemase activity. However, several microorganisms produce chromosomally mediated zinc-β-lactamases that inactivate carbapenems, e.g., imipenem, biapenem, and meropenem. Their function is based more on a zinc-dependent mechanism than on the 'classical' serine-ester mechanism of other β-lactamases. These enzymes usually consist of one single subunit with a molecular weight ranging between 25,000–35,000 Da. The most frequent microorganisms producing zinc-β-lactamases are *Stenotrophomonas maltophilia, Aeromonas hydrophila, Bacillus cereus, Flavobacterium odoratum*, *Bacteroides fragilis,* and *Legionella gormani* [37, 38]. More threatening is the report of a plasmid-encoded zinc-dependent carbapenemase in *P. aeruginosa* and *Serratia marcescens*, two major nosocomial pathogens [39–40].

Antimicrobial spectrum

Due to its unique structure, its good penetration through the outer membrane of gram-negative bacteria and its high affinity for PBPs, meropenem is effective against the majority of human bacterial pathogens. Based on the minimal inhibitory concentration (MIC), the activity of meropenem against microorganisms is summarized in table 1.

Tolerability and clinical profile

Meropenem has an excellent toxicity profile with a pattern and frequency of adverse events similar to other β-lactam antibiotics. The most frequently reported adverse events have been diarrhea (1.9%), rash (1%), nausea (1%), thrombocytosis (1.9%), eosinophilia (0.9%), and changes in hepatic enzymes (4–5%). The low incidence of seizures (0.38%) and its good tolerability at high doses make meropenem useful for the treatment of meningitis, where high doses are required [41].

The neurotoxicity of β-lactams is related to their ability to bind to the receptor of γ-aminobutyric acid (GABA), an inhibitory neurotransmitter in the central nervous system. Inhibition of GABA activity leads to increased electrical activity in the brain and to seizures [42]. The different ability of β-lactams to interfere with GABA binding has been demonstrated in a mouse GABA receptor-binding assay by determining the IC_{50}. The IC_{50} represents the amount of drug required to inhibit GABA receptor binding by 50%. The IC_{50} of meropenem is 20 times higher than that of imipenem (20 mM for meropenem versus 1 mM for imipenem), indicating that meropenem has a very low affinity for the GABA receptor. Accordingly, the dose of meropenem needed to induce seizures in mice is 20 times higher than for imipenem (>300 nmol for meropenem versus 14 nmol for imipenem) [43].

The ultra-broad spectrum of antibacterial activity and high bactericidal efficacy make meropenem suitable for the empirical treatment of severe bacterial infections, in particular lower respiratory tract infections, intra-abdominal infections, gynecological infections, septicemia in non-neutropenic and neutropenic patients, and meningitis.

Table 1. Activity of Meropenem against selected clinically important bacteria.

Organisms	Number	MIC_{50} (mg/l)	MIC_{90} (mg/l)
Gram-positive aerobes			
Methicillin-susceptible staphylococci			
Staphylococcus aureus	5258	0.13	0.25
Staphylococcus epidermidis	1431	0.25	4
Methicillin resistant staphylococci			
Staphylococcus aureus	608	4	32
Staphylococcus epidermidis	352	4	16
Streptococcus pyogenes	486	0.008	<0.06
Streptococcus pneumoniae (PenS)	1442	0.016	0.13
Streptococcus pneumoniae (PenR)	272	0.5	1
Enterococcus faecalis	2222	4	8
Fastidious strains			
Haemophilus influenzae	1829	0.06	0.13
Neisseria meningitidis	171	0.008	0.03
Moraxella catarrhalis	386	<0.008	0.008
Gram-negative aerobes			
Escherichia coli	7658	0.03	<0.06
Citrobacter freundii	1330	<0.06	0.13
Klebsiella pneumoniae	2911	<0.06	0.06
Enterobacter cloacae	2590	0.06	0.25
Serratia marcescens	1619	0.06	0.25
Proteus mirabilis	2551	0.06	0.13
Non-fermenters			
Acinetobacter calcoaceticus	589	0.5	2
Pseudomonas aeruginosa	5946	0.5	4
Burkolderia cepacia	433	2	8
Anaerobes			
Bacteroides fragilis	1868	0.13	0.5
Veillonella parvula	41	0.03	0.13
Peptostreptococcus anaerobius	174	0.25	1
Peptostreptococcus magnus	151	0.13	0.25
Clostridium perfringens	462	0.08	<0.06
Clostridium difficile	256	1	2

PenS/R; penicillin-susceptible/resistant; MIC50/90: concentrations needed to inhibit the growth of 50/90% of the strains [AstraZeneca, personal communication].

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