Mechanisms of Action

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ANTIBIOTICS THAT ACT ON THE CELL WALL

The basis of the bacterial cell wall is peptidoglycan, a polymer that contains alternating residues of glucosamine and muramic acid in β-1→4 linkage. The carboxyl groups of muramyl residues are substituted by short peptides (usually pentapeptides such as L-Ala–D-Glu–D-Asp–D-Ala–D-Ala, L-Ser–D-Glu–D-Asp–D-Ala–D-Ala, or Gly–D-Glu–D-Asp–D-Ala–D-Ala). Cell-wall-active antibiotics act by inhibiting the activity of enzymes involved in the synthesis of the precursors or in the reticulation of peptidoglycan.

BETA-LACTAMS

The beta-lactam nucleus is the basic building block of an exceptionally large class of antibiotics, all of which share a common mode of action but have quite distinct properties in terms of spectrum, pharmacokinetics and activity against resistant strains (see Chapter 7.5).

Chemical structure

All antibiotics in this class contain a cyclic amide called beta-lactam, but different classes have been described according to the nature of the cycle or of the heteroatom included in the cycle. The main classes are (Fig. 1.1):

- penams – beta-lactams with a five-membered ring containing a sulfur atom (penicillins);
- clavams – beta-lactamase inhibitors that contain a five-membered ring with an oxygen heteroatom (e.g. clavulanic acid; some sulfur analogs have also been reported);
- carbapenems – five-membered rings with a double bond (e.g. thienamycin, imipenem);
- cephems – six-membered unsaturated rings with a sulfur atom (cephalosporins);
- oxacephems – the oxygen analogs of cephems (latamoxef); and
- monobactams – cyclic amides in a four-membered ring (azetidine) with a methylcarboxylate function in the case of nocardicins and a sulfonate in the case of the other monobactams (e.g. aztreonam).

Other representative members of the beta-lactams are thiacephems, dethiacephems, dethiacephams, heterocephems and cephams, as well as diverse bicyclic systems. Some non-beta-lactam analogs have also been reported but they seem to be of little interest.

Mode of action

Beta-lactams act primarily as inhibitors of the synthesis of the cell wall, by blocking the action of transpeptidases (Fig. 1.2).[1]

Specialized acyl serine transferases or transpeptidases are involved in the assembly of the bacterial cell wall. The structural properties of beta-lactams mimic the D-Ala–D-Ala sequence in that the distance between the carboxylate and the cyclic amide is similar. Thus, these antibiotics act as a false substrate for D-alanyl-D-alanyl transpeptidases. The carboxylate or the sulfonate group of the beta-lactams reacts with a serine residue of the transpeptidases [also called penicillin-binding proteins (PBPs)] to give an acyl enzyme, with the formation of a covalent bond (Fig. 1.3).[2] The acylated enzyme is inactive. Such a mechanism is called suicide inhibition or mechanism-based enzyme inactivation. Transpeptidases are located in the periplasmic space, which is directly accessible in Gram-positive bacteria. In Gram-negative bacteria, beta-lactams have to cross the outer membrane of the bacteria either through the membrane (by passive diffusion) or via porin channels.

The perturbations induced by the beta-lactams in cell wall formation explain the inhibition of growth of the bacteria, but the bactericidal effect results from indirect mechanisms (mostly the activation of autolytic enzymes). Beta-lactams are active only against highly dividing bacteria.

Resistance

Resistance to beta-lactams may occur at three different levels (see Chapter 7.2). First, access to the PBPs in Gram-negative bacteria might be abolished by an alteration of porin channels. This phenomenon predominantly affects highly water-soluble beta-lactams. Second, modification of PBPs can also be observed, particularly of PBP2, which is an essential protein involved in the 'shaping' of the

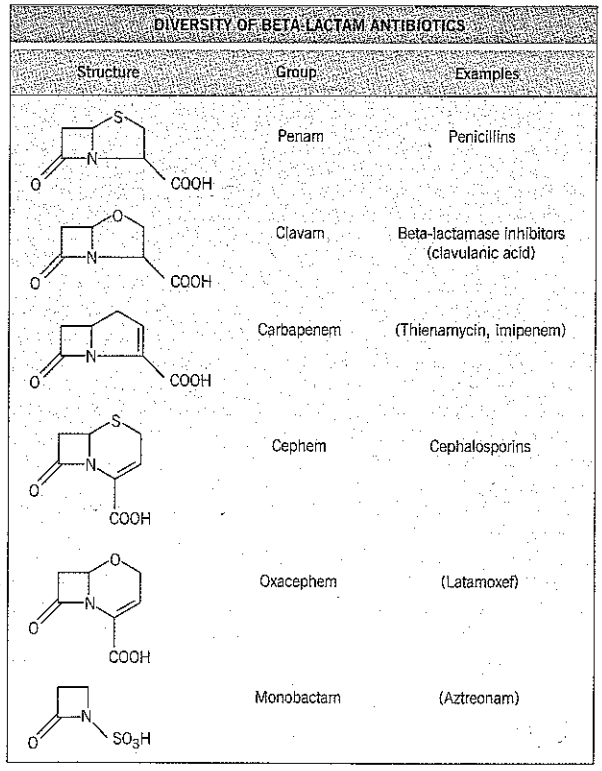


Fig. 1.1 Diversity of beta-lactam antibiotics. Main ring structures names and representative antibiotics.

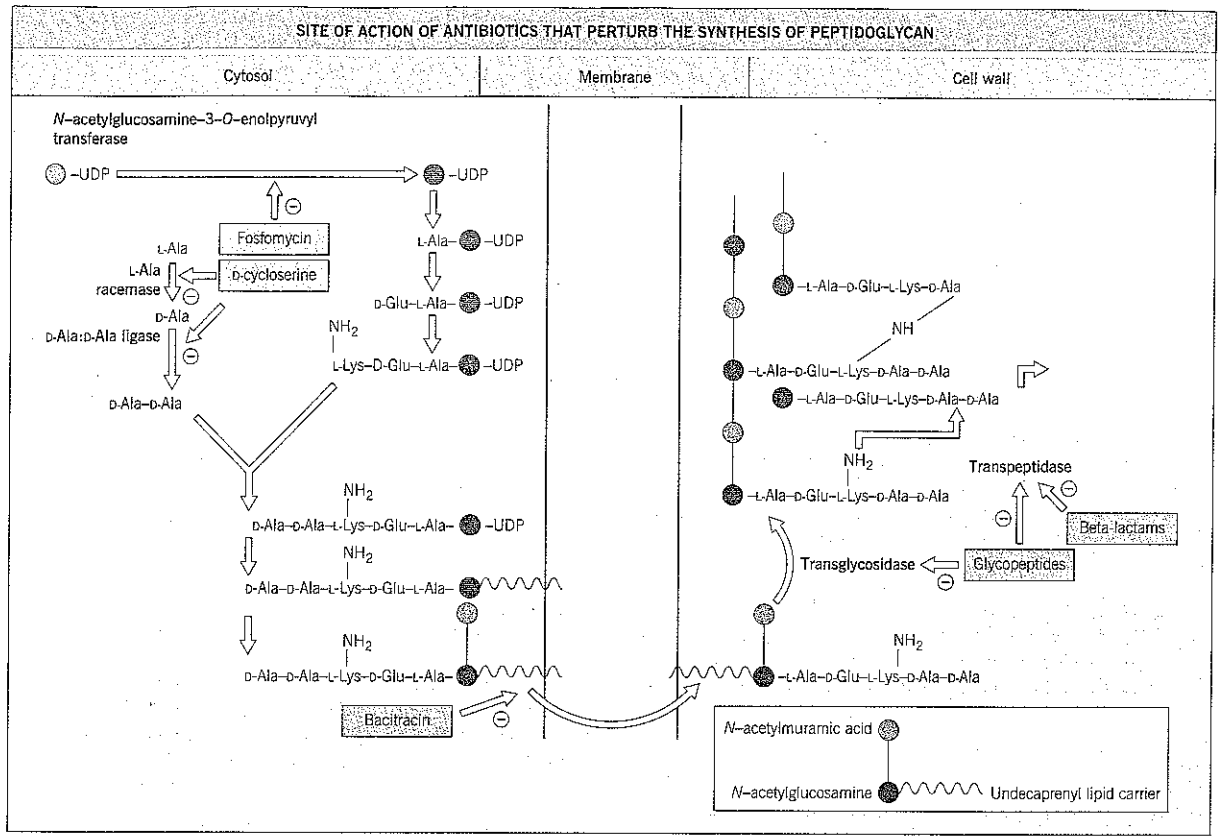


Fig. 1.2 Site of action of antibiotics that perturb the synthesis of peptidoglycan. The peptidoglycan unit is formed in the cytosol of the bacteria, by binding to uridine diphosphate (UDP)-*N*-acetylmuramic acid of a short peptide (the nature of which differs from one bacteria to the next). This precursor is then attached to a lipid carrier and added to *N*-acetylglucosamine before crossing the bacterial membrane. At the cell surface, peptidoglycan units are reticulated by the action of transglycosylases (catalyzing the polymerization between sugars) and of transpeptidases (catalyzing the polymerization between peptidic chains). The antibiotics act as follows: fosfomycin is an analog of phosphoenolpyruvate, the substrate of the *N*-acetylglucosamine-3-*o*-enolpyruvyl transferase synthesizing *N*-acetylmuramic acid from *N*-acetylglucosamine and phosphoenolpyruvate; cycloserine is an analog of D-Ala, and blocks the action of D-Ala racemase and D-Ala:D-Ala ligase; bacitracin inhibits the transmembrane transport of the precursor; vancomycin binds to D-Ala–D-Ala termini and thus inhibits the action of transglycosylases and transpeptidases; and beta-lactams are analogs of D-Ala–D-Ala and suicide substrates for transpeptidases.

bacteria. Resistant strains (methicillin-resistant staphylococci) produce a PBP2 protein with a very low affinity for beta-lactams. Other PBPs can also show the same decreased affinity.

The third and most abundant mechanism is the production of hydrolyzing enzymes called beta-lactamases.[2,3,4,5] These enzymes are serine protease enzymes that cleave the beta-lactam ring by opening the amide bond. The corresponding genes may either be carried on chromosomes (and their expression may be constitutive or inducible) or on plasmids. This system of resistance is very efficient as these enzymes are secreted out of the cell wall in Gram-positive bacteria and in the periplasmic space in Gram-negative bacteria, and the affinity for beta-lactams is greater than that for PBPs. Most beta-lactamases open the beta-lactam ring exactly as do transpeptidases, but the major difference is that the hydrolysis rate is by far quicker in the case of beta-lactamase than in the case of PBP (see Fig. 1.3). In other words, the speed of hydrolysis of the acyl enzyme is higher and explains the high efficiency of beta-lactamases. The turnover of PBPs and beta-lactamases is indeed very different (1 beta-lactam per hour and 1000 beta-lactams per second respectively). Analytical data and genetic studies of beta-lactamases and PBPs show a high level of structural homology, which suggests that both derive from a common ancestor. A number of beta-lactams have been made resistant to beta-lactamases by appropriate steric hindrance or change in conformation (Fig. 1.4), giving rise to the large number of successive generations of penicillins and cephalosporins. Beta-lactamases, however, have an extraordinary plasticity and inevitably develop activity against all new derivatives at a fast pace (Fig. 1.5).

Thanks to their specific structure, clavams are poor antibiotics but bind tightly to beta-lactamases. Given in combination with beta-lactams, they provide protection unless the bacteria produce beta-lactamases. Some beta-lactamases can also hydrolyze clavams.

Pharmacodynamics

Beta-lactams are relatively slow-acting antibiotics and must be present at a concentration above the MIC as long as possible, although achieving concentrations more than 4–5 times the MIC provides little gain in activity so that frequent dosing is more appropriate than infrequent administration of large doses. In general, beta-lactams show only a moderate postantibiotic effect.

GLYCOPEPTIDES

Chemical structure

Glycopeptide antibiotics (vancomycin, teicoplanin) contain two sugars

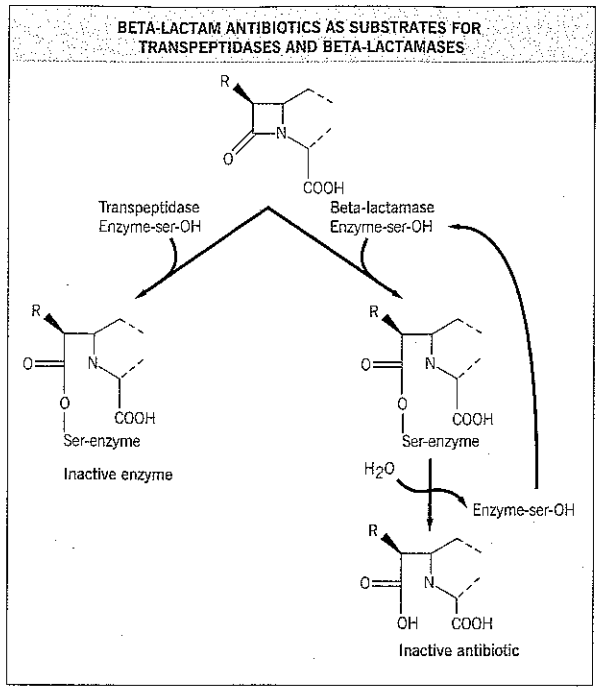


Fig. 1.3 Beta-lactam antibiotics as substrates of transpeptidases and beta-lactamases. The left part of the figure shows how a beta-lactam covalently binds to the transpeptidase. The hydrolysis of this acylated enzyme is very slow (1 beta-lactam per hour), making the enzyme inactive. The right part of the figure shows that the same reaction occurs in the case of a beta-lactamase. The hydrolysis of the acyl-enzyme is now very rapid (1000 beta-lactams per second), making the antibiotic inactive and regenerating the enzyme for a new cycle of hydrolysis.

and an aglycone moiety made of a relatively highly conserved heptapeptide core, in which two amino acids bear a chloride substituent (see Chapter 7.10). The aglycone fraction is responsible for the pharmacologic activity of the molecule, whereas the sugars are thought to modulate its hydrophilicity and its propensity to form dimers (see below). As a result of their large size, glycopeptides are not only unable to cross the outer membrane of Gram-negative bacteria (which explains their inactivity against these organisms), but are also unable to penetrate inside bacteria, which limits them to an extracellular target.

Mode of action

Glycopeptide antibiotics inhibit the late stages of cell wall peptidoglycan synthesis (see Fig. 1.2). Glycopeptides bind to D-Ala–D-Ala termini of the pentapeptide-ending precursors localized at the outer surface of the cytoplasmic membrane. At the molecular level, glycopeptides form a high affinity complex with D-Ala–D-Ala by establishing hydrogen bounds via their aglycone moiety.[6] The strength of this binding is, however, greatly enhanced either by dimerization of the glycopeptide molecules (mediated by their sugars and the chloride atoms substituents on the aglycone – as observed in vancomycin) or by anchoring of glycopeptide molecules in the membrane by a fatty acyl chain substituent (as observed in teicoplanin).[7] The subsequent steric hindrance around the pentapeptide termini blocks the reticulation of peptidoglycan by inhibiting the activity of transglycosylases (responsible for the new disaccharide–pentapeptide subunit on the nascent peptidoglycan) and of transpeptidases (catalyzing the formation of interpeptide bridges).[6]

Resistance

Resistance to glycopeptides results from substituting a *d*-lactic acid in place of terminal D-Ala of the pentapeptide. Although this does not prevent the action of the transpeptidase, it prevents the binding of the glycopeptides because of the loss of one crucial hydrogen bond.[8]

Pharmacodynamics

Glycopeptide antibiotics show a very slow bactericidal activity, which is not very dose-dependent, for reasons that are unclear. It has been proposed that their inhibition of cell wall synthesis blocks the growth of bacteria and therefore the synthesis of DNA, RNA and proteins, whereas the autolytic enzymes could continue to function. As their activity is time-dependent, glycopeptides need repeated administration (continuous infusion has even been proposed and is being evaluated clinically for severe infections). In this respect, their moderate (2 hours) postantibiotic effect could be advantageous.

Glycopeptides show, at least *in vitro*, a synergic effect with aminoglycosides, probably by facilitating the penetration of these polar molecules into bacteria.

Future developments

New derivatives with an hydrophobic substituent (e.g. LY333328) act against vancomycin-resistant strains and show a fast and concentration-dependent bactericidal effect, which suggests a distinct mode of action that could involve drug dimerization and membrane destabilization.[9]

OTHER AGENTS THAT ACT ON CELL WALL SYNTHESIS

D-Cycloserine is a broad-spectrum antibiotic active through its similarity with D-Ala (see Figs 1.2 & 1.6), inhibiting the conversion of L-Ala into D-Ala (reaction catalyzed by a racemase) and also the dimerization of the D-Ala (reaction catalyzed by the D-Ala:D-Ala ligase).[10]

Fosfomycin, which bears structural similarities to phospho-*enol*-pyruvate, inhibits a very early stage of peptidoglycan synthesis by impairing the formation of uridine diphosphate (UDP)–*N*-acetylglucosamine-*enol*-pyruvate, a precursor of UDP–*N*-acetylmuramic acid (see Figs 1.2 & 1.6).[11]

Bacitracin is a polypeptide of complex structure. It acts as an inhibitor of the peptidoglycan synthesis at the level of translocation of the precursor across the bacterial membrane (see Fig. 1.2).[12]

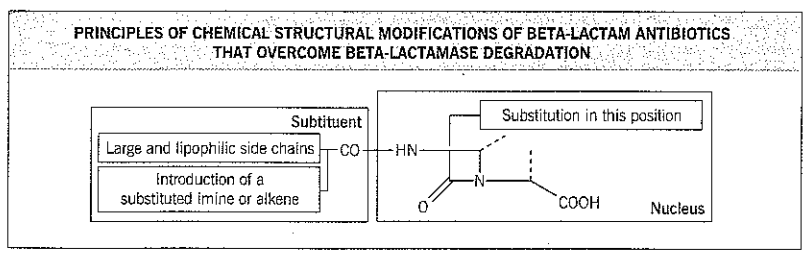


Fig. 1.4 Structural modifications of beta-lactam antibiotics in order to overcome beta-lactamase degradation. A first strategy, applied in penicillins, cephalosporins, oxacephems, and monobactams, consists of the introduction of a large side chain on the nucleus, possibly containing a substituted imine or alkene. A second strategy, applied in oxacephems and cefoxitin, consists of the introduction of a methoxy group on the beta-lactam ring.

FUNCTIONAL CLASSIFICATION OF BETA-LACTAMASES				
Group	Molecular class	Preferred substrates	Active beta-lactams	Typical examples
Group 1: serine cephalosporinases not inhibited by clavulanic acid	C	Cephalosporins I and II (>> cephalosporins III, monobactams, penicillins)	Carbapenems Temocillin (Cephalosporins III and IV, variable upon level of expression)	AmpC from Gram-negative; variable upon the species
Group 2: serine beta-lactamases				
2a: penicillinases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbenicillin >> oxacillins)	Amoxicillin + clavulanic acid Cephalosporins Carbapenems	Penicillinases from Gram-positive
2b: broad-spectum beta-lactamases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbencillin >> oxacillins) Cephalosporins I and II	Cephalosporins III and IV, Monobactams* Carbapenems Amoxicillin + clavulanic acid	TEM-1, TEM-2, SHV-1 from Enterobacteriacea, *Haemophilus* spp. *Neisseria gonorrhoeae*
2be: extended-spectrum beta-actamases inhibited by clavulanic acid (ESBL)	A	Penicillins, Cephalosporins I II III (IV) Monobactams	Carbapenems Temocillin	TEM-3 to -26 from Enterobacteriacea SHV-2 to -6 from *Klebsiella* spp. K1-OXY from *Klebsiella oxytoca*
2br: broad-spectrum beta-lactamases with reduced binding to clavulanic acid	A	Penicillins	Most cephalosporins Monobactams* Carbapenems	TEM-30 to -41 (=IRT-1 to IRT-12) from *Escherichia coli*
2c: carbenicillin-hydrolyzing beta-lactamases generally inhibited by clavulanic acid	A	Penicillins Carbenicillin (Cephalosporins I and II)	Piperacillin + tazobactam Cephalosporins III and IV Monobactams* Carbapenems	PSE-1, PSE-3, PSE-4 from *Pseudomonas aeruginosa*
2d: cloxacillin-hydrolyzing beta-lactamaxes generally inhibited by clavulanic acid	D	Penicillins Cloxacillin Cephalosporins I and II	Carbapenems Cephalosporins III Monobactams* Piperacillin + tazobactam	OXA-1 to -11, PSE-2 from Enterobacteriacea and *Pseudomonas aeruginosa*
2e: cephalosporinases inhibited by clavulanic acid	A	Cephalosporins I and II	Cephalosporins III and IV Monobactams* Penems	FPM-1 from *Proteus vulgaris* Cep-A from *Bacteroides fragilis*†
2f: carbapenem-nonmetallo-hydrolyzing beta-lactamases	A	Penicillins Cephalosporins Carbapenems	(Cephalosporins III and IV) (Monobactams*)	NMC-A, IMI-1 from *Enterobacter cloacae* Sme-1 from *Serratia marcescens*
Group 3: Metallo-beta-lactamases inhibited by EDTA	B	Most beta-lactams, including carbapenems	Monobactams*‡	L-1, XM-A from *Stenotrophomonas maltophilia* CcrA from *Bacteroides fragillis* A2h, CphA from *Aeromonas hydrophila* IMP-1 in *Pseudomonas* spp. and *Serratia* spp.
Group 4: Penicillinases not inhibited by clavulanic acid		Penicillins, including carbenicillin and oxacillin	Monobactams*†‡ and generally carbapenems	SAR-2 from *Pseudomonas cepacia*

*Monobactams are not active on Gram-positive bacteria
†Penems are the only molecules active in this case
‡Remain active for most of the rare published studies

Fig. 1.5 Functional classification of beta-lactamases. The number of enzymes as well as their spectrum of activity is continually evolving. Data from Bush *et al.*, 1995.[5]

ANTIBIOTICS THAT ACT ON PROTEIN SYNTHESIS

Bacterial ribosomes comprise a 30S subunit, which binds mRNA and initiates the protein synthesis, and a 50S subunit, which binds aminoacyl tRNA, catalyzes the peptide bond formation and controls the elongation process. The main sites identified in the ribosome are the donor peptidyl site (P-site), where the growing peptide chain is fixed, and the acceptor aminoacyl site (A-site), where peptide bond formation occurs.

AMINOGLYCOSIDES

Chemical structure

Streptomycin was discovered in 1944 but this compound has a limited spectrum of activity (see Chapter 7.7). Several other compounds with a broader spectrum of activity than streptomycin toward aerobic and facultative Gram-negative bacilli were extracted from bacteria or semisynthesized over the subsequent 20 years (aminoglycosides from kanamycin or gentamicin families). In the 1970s, netilmicin and amikacin demonstrated the possibility of developing compounds active against strains resistant to earlier aminoglycosides.

Aminoglycosides are made of several aminated sugars joined by glycosidic linkages to a dibasic cyclitol.[13] The latter is streptidine in streptomycin and derivatives, fortamine in the fortimicin series, and 2-deoxystreptamine in most aminoglycosides used clinically. The 2-deoxystreptamine moiety links to cyclic sugars either at positions 4 and 5 (neomycin and paromomycin) or 4 and 6 (kanamycin, tobramycin, amikacin and dibekacin in the kanamycin family; gentamicin C_1, C_{1a}, C_2 and isepamicin in the gentamicin family, sisomicin and netilmicin) (Fig. 1.7). All compounds are positively charged at physiologic pH.

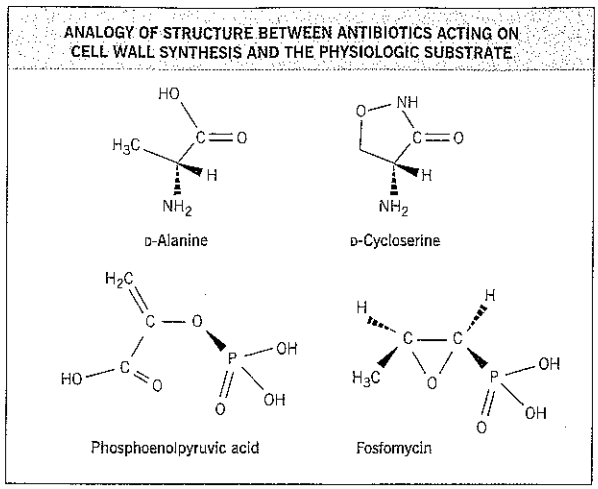


Fig. 1.6 Analogy of structure between antibiotics acting on cell wall synthesis and the physiologic substrate. The two antibiotics act as analogs of the corresponding substrate.

Bacterial targeting

Aminoglycosides selectively disturb the protein synthesis of bacteria because they bind to the 30S subunit of the bacteria's ribosomes, which does not exist in eukaryotic cells. However, molecules that display an hydroxyl function at C6′ in place of an amino function also affect protein synthesis in cultured mammalian cells, as do high doses of gentamicin, probably through nonspecific binding to ribosomes and/or nucleic acids.

Mode of action

As a result of their highly polar character, aminoglycosides are unable to diffuse through membranes, and therefore require specific mechanisms of transport. Their passage across the outer membrane of Gram-negative bacteria occurs by a process that is not energy dependent and involves the drug-induced disruption of Mg^{2+} bridges between adjacent lipopolysaccharide molecules. By contrast, their transport across the cytoplasmic (inner) membrane is dependent upon electron transport, and is therefore termed energy-dependent phase I [EDP-I]. The greater the transmembrane electrical potential, the greater the antibacterial effect of the aminoglycoside. In an anaerobic environment, at low external pH and in highly osmolar culture media, this transmembrane electrical potential is decreased, which explains the reduction of antibacterial activity observed.

Once in the bacterial cytosol, aminoglycosides bind to the aminoacyl site of the 30S subunit of ribosomes[14] (and, to a lesser extent, to specific sites of the 50S subunit), again through an energy-dependent process (EDP-II), disturbing the elongation of the nascent peptide. Their mechanism of action is complex, involving inhibition of the transfer of the peptidyl tRNA from the A-site to the P-site and impairment of the proofreading process that controls translational accuracy. The latter action leads to misreading and/or premature termination in protein synthesis. The final effects vary somewhat from one compound to another, which possibly explains differences in the killing rates. The aberrant proteins may be inserted into the cell membrane, which results in altered permeability and further stimulation of aminoglycoside transport.

Resistance

Resistance occurs mostly by the production of enzymes that inactivate the functions responsible for activity (Fig. 1.8; see Chapter 7.2). Semisynthetic derivatives (netilmicin, amikacin, isepamicin, etc.) were therefore made specifically to afford protection against the main enzymes. However, whereas previously resistant bacteria harbored only one of a very few types of enzymes, the production of several enzyme types is increasingly more common, causing multiple resistance. It is believed that most of these enzymes have physiologic effects on natural substrates and act on aminoglycosides only opportunistically in the initial introduction of these antibiotics. However, point mutations and selection may have quickly increased their specificity and efficacy.[16]

A second mechanism of resistance is membrane impermeability, which confers resistance to all aminoglycosides. Its molecular mechanism is unclear.

Pharmacodynamics

Aminoglycosides demonstrate a rapid, concentration-dependent bactericidal effect and an important postantibiotic effect (probably because of a largely irreversible binding to the ribosomes). Therefore the optimal mode of administration of these antibiotics is once-a-day, allowing elevated serum concentrations to be reached.

Aminoglycosides show synergistic activity with antibiotics that act on cell wall synthesis, because they facilitate the penetration of aminoglycosides into the bacteria. In contrast, their activity is antagonized by bacteriostatic agents such as chloramphenicol and tetracyclines, probably by inhibition of their energy-dependent uptake and by interference with the movement of the ribosome along mRNA.

Future developments

Efforts are being undertaken in two directions:

- to increase the binding affinity while retaining binding selectivity; and
- to develop, taking into account the biochemical, geometric and pharmacokinetic knowledge of aminoglycoside-inactivating enzymes, new aminoglycoside derivatives resistant to these enzymes.

Although some derivatives have been made by pharmacochemical approaches, little success has resulted. A most innovative approach could be to use our understanding of the aminoglycoside-inactivating enzymes to produce either totally new aminoglycoside derivatives or enzyme inhibitors.

TETRACYCLINES

Chemical structure

The first tetracyclines discovered were isolates from *Streptomyces* spp. (tetracycline, oxytetracycline), whereas more recent long-acting compounds (doxycycline, minocycline) are semisynthetic. All such molecules contain four hydrophobic fused rings, which are diversely substituted, but principally by oxygenated hydrophilic groups (see Chapter 7.11).

Bacterial targeting

Tetracyclines penetrate the outer membrane of Gram-negative organisms through porins. Accumulation inside the bacteria depends on the pH gradient between the cytosol and the external medium, but it is unclear whether transmembrane transport occurs by diffusion or via a proton-driven carrier. The main argument in favor of the latter is that it could explain the selective action of tetracyclines by preferential transport in the bacterial cells (Fig. 1.9).

Mode of action

Tetracyclines interfere with the initiation step of protein synthesis (see Fig. 1.9). More precisely, they inhibit the binding of aminoacyl tRNA to the A-site of the ribosome. The 7S protein and 16S RNA show the best affinity for tetracyclines, and are therefore the main targets involved pharmacologically.[17] This binding inhibits the fixation of a new aminoacyl tRNA on the ribosome. At higher concentrations, tetracyclines also bind to the 23S RNA, which is part of the peptidyl

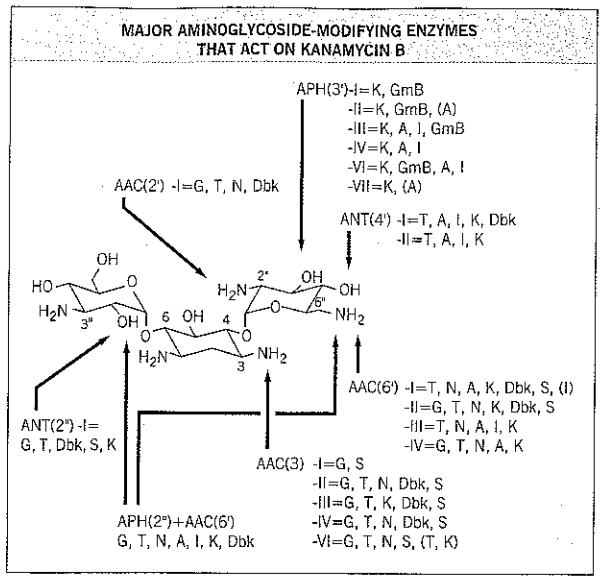


Fig. 1.7 Major aminoglycoside-modifying enzymes that act on kanamycin B. (This aminoglycoside is susceptible to the largest number of enzymes.) The *N*-acetyltransferases (AAC) affect amino functions and the *o*-nucleotidyltransferases (ANT) and the *o*-phosphotransferases (APH) affect the hydroxyl functions. Each group of enzymes inactivates specific sites, but each of these sites can be acted upon by distinct isoenzymes (roman numerals) with different substrate specificities (phenotypic classification). At least one enzyme is bifunctional and affects both positions 2" (*o*-phosphorylation) and 6' (*N*-acetylation). The main clinically used aminoglycosides on which these enzymes act are as follows: amikacin (A), dibekacin (Dbk), commercial gentamicin (G), gentamicin B (Gmb), kanamycin A (K), isepamicin (I), netilmicin (N), sisomicin (S) and tobramycin (T). The drug abbreviations that appear in parentheses are those for which resistance was detectable *in vitro* even though clinical resistance was not conferred. Data from Shaw *et al.*, 1993.[15]

transferase region of the ribosome. However, the enzymatic activity of this site does not seem to be disturbed by tetracyclines. Additional actions on ribosomal functions have been proposed:[18]

- tetracyclines bind, or at least protrude, in the P-site, thanks to the change in ribosome conformation in the post-translocational state; and
- tetracyclines modify the ribosome conformation at the head of the 30S subunit and at the interfacial side of the 50S subunit.

More recently, it has been shown that tetracyclines have chondroprotective effects in inflammatory arthritis models, an action related to their ability to inhibit the expression of nitric oxide synthases induced by inflammatory conditions.[19] Clinical evaluation is, however, needed to document further the potential usefulness of tetracyclines as modulators of the inflammatory response. A possible application would be the treatment of arthritis of bacterial origin (e.g. Lyme disease).

Resistance

Resistance to tetracyclines is now widespread, and is related to a decrease in the bacterial drug content caused by an active drug efflux (see Fig. 1.9).[20] Efflux mechanisms appear much more important and are somehow largely, but not entirely unrelated to the drug structure (see fluoroquinolones and macrolides below; see Chapter 7.2).

Pharmacodynamics

Tetracyclines are essentially bacteriostatic and must be administered at intervals short enough, in terms of the drug half-life, to maintain their serum level above the infecting organism's MIC for as long as possible.

Future developments

Glycylcyclines are tetracycline derivatives that bear a glycyl substituent and are able to bind to the tetracycline binding site on the ribosome.[21] Their main advantage is that they conserve activity against strains with acquired resistance to conventional tetracyclines by the production of efflux pumps or by a mechanism of ribosomal protection (acquisition of a gene that encodes a Tet protein, i.e. an elongation factor able to displace the tetracycline bond on the ribosome). However, mutants resistant to glycylcyclines have already been selected *in vitro*, with the possibility that such mutants may emerge in clinical strains.

FUSIDIC ACID

Chemical structure

Fusidic acid is a steroid-like structure, a member of the fusidane class. It is used in its sodium salt form.

Mode of action

Fusidic acid prevents the dissociation of the complex that is formed by guanosine diphosphate, the elongation factor 2 and the ribosome. It thereby inhibits the translocation step of peptidyl-tRNA from the P site to the A site of the ribosome and, therefore, the elongation of the nascent polypeptide chain.

Pharmacodynamics

Fusidic acid is bacteriostatic but it may be bactericidal at high concentrations.

MUPIROCIN

Chemical structure

Mupirocin contains a short fatty acid side chain (9-hydroxynonanoic acid) linked to monic acid by an ester linkage. Mupirocin is also called pseudomonic acid because its major metabolite is derived from submerged fermentation by *Pseudomonas fluorescens*. Pseudomonic acid A is responsible for most of the antibacterial activity; three other minor metabolites of similar chemical structure and antimicrobial spectrum are called pseudomonic acids B, C and D.

Mode of action

Mupirocin inhibits bacterial RNA and protein synthesis by binding to bacterial isoleucyl tRNA synthetase, which catalyzes the formation of isoleucyl tRNA from isoleucine and tRNA. This prevents incorporation of isoleucine into protein chains, and so halts protein synthesis. This unique mechanism of action results in no cross-resistance between mupirocin and other antimicrobial agents.[22]

Pharmacodynamics

Mupirocin is bacteriostatic at low concentrations, but becomes bactericidal at concentrations achieved locally by topical administration. In-vitro antibacterial activity is greatest at acidic pH, which is advantageous in the treatment of cutaneous infections, because of the low pH of the skin.

MACROLIDES

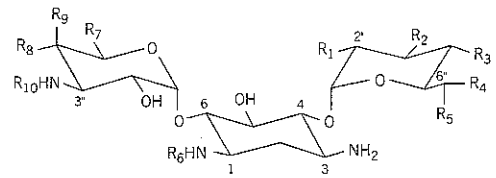
Chemical structure

The main active macrolides are 14-, 15-, or 16-membered lactone rings, substituted by two sugar moieties, of which one bears an aminated function (see Chapter 7.6). In 15-membered macrolides (azithromycin), an additional amino function is inserted in the lactone ring, conferring to this subclass of molecule the name 'azalides' (Fig. 1.10).

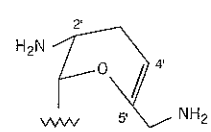
Erythromycin, the first clinically developed macrolide, is a natural product. Most of the more recent molecules are semisynthetic derivatives thereof, designed to be stable in acidic conditions, and as such are characterized by an improved oral bioavailability. 16-Membered macrolides are intrinsically acid stable.

STRUCTURAL FORMULAE OF THE 2-DEOXYSTREPTAMINE-CONTAINING AMINOGLYCOSIDES

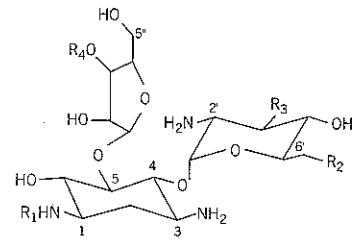
4, 6-Disubstituted deoxystreptamine



(a)



4, 5-Disubstituted deoxystreptamine



	Aminoglycoside	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}
Kanamycins	Kanamycin A	OH	OH	OH	H	NH_2	H	CH_2OH	OH	H	H
	Kanamycin B	NH_2	OH	OH	H	NH_2	H	CH_2OH	OH	H	H
	Kanamycin C	NH_2	OH	OH	H	OH	H	CH_2OH	OH	H	H
	Amikacin	OH	OH	OH	H	NH_2	COR′	CH_2OH	OH	H	H
	Tobramycin	NH_2	H	OH	H	NH_2	H	CH_2OH	OH	H	H
	Dibekacin	NH_2	H	H	H	NH_2	H	CH_2OH	OH	H	H
	Arbekacin*	NH_2	H	H	H	NH_2	COR′	CH_2OH	OH	H	H
Gentamicins	Gentamicin C_1†	NH_2	H	H	CH_3	$NHCH_3$	H	H	CH_3	OH	CH_3
	Gentamicin C_{1a}†	NH_2	H	H	H	NH_2	H	H	CH_3	OH	CH_3
	Gentamicin C_2†	NH_2	H	H	CH_3	NH_2	H	H	CH_3	OH	CH_3
	Gentamicin C_{2b}†	NH_2	H	H	H	$NHCH_3$	H	H	CH_3	OH	CH_3
	Gentamicin B†	OH	OH	OH	H	NH_2	H	H	CH_3	OH	CH_3
	Isepamicin†	OH	OH	OH	H	NH_2	H	H	CH_3	OH	CH_3
	Sisomicin	–	–	–	–	–	H	H	CH_3	OH	CH_3
	Netilmicin	–	–	–	–	–	CR″	H	CH_3	OH	CH_3

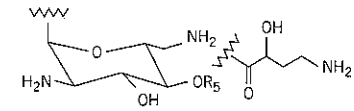
R=$CHOHCH_2NH_2$; R′=$CHOH(CH_2)_2NH_2$; R″=CH_2CH_3 (a)=primed sugar for sisomicin and netilmicin

*In Japan

†Commercially available gentamicin is a mixture of C1, C1a and C2 in a ratio of 30, 30 and 40%, respectively

Aminoglycoside	R_1	R_2	R_3	R_4	R_5
Neomycin B	H	NH_2	OH	X	H
Paromomycin I	H	OH	OH	X	H
Lividomycin A	H	OH	H	X	Mannose
Ribostamycin	H	NH_2	OH	H	
Butirosin B	Y	NH_2	OH	H	

X=



Y=

Fig. 1.8 Structural formulae of the 2-deoxystreptamine-containing aminoglycosides. In the numbering of the atoms, the primed numbers are ascribed to the sugar attached to the C-4 of the 2-deoxystreptamine (as this carbon atom is of the R configuration) and the doubly primed numbers are ascribed to the sugar attached on eithre the C-6 (S configuration) for the 4,6-disubstituted-2-deoxystreptamine or the C-5 (R configuration) for the 4,5-disubstituted-2-deoxystreptamine. Molecules indicated in bold denote the aminoglycosides in common clinical use.

Bacterial targeting

Macrolides specifically bind to the 50S subunit of the ribosomes, which does not exist in eukaryotic cells.

Mode of action

Macrolides reversibly bind to the peptidyl transferase center, located at the 50S surface, which results in multiple alterations of the 50S subunit functions.[23] Macrolides are classically thought to block the peptide bond formation or the peptidyl tRNA translocation from the A- to the P-site. However, additional consequences of macrolides binding to ribosomes have been reported. A proposal is that they could also favor the premature dissociation of peptidyl tRNA from the ribosome during the elongation process, leading to the synthesis of incomplete peptides.[24] A further suggestion is that erythromycin prevents the assembly of the 50S subunit, but this does not appear to be applicable to other macrolides.

Resistance

Clinically meaningful resistance occurs primarily by modification of the bacterial target and therefore affects all macrolides (and will also affect lincosamides and streptogramins). This resistance may be inducible or constitutive. 16-Membered macrolides are not inducers and therefore show activity on a subset of resistant strains. Efflux mechanisms are also now being observed and, again, 16-membered macrolides are spared this effect. The frequency of strains susceptible to 16-membered macrolides and resistant to 14- and 15-membered macrolides remains small, however.

Pharmacodynamics

Macrolides are essentially bacteriostatic antibiotics, except at high concentrations. Thus, their concentration at the infected site needs to be consistently maintained above the MIC of the pathogen.

As their mode of action is similar, macrolides, streptogramins, lincosamides and chloramphenicol have antagonistic pharmacologic activity. Moreover, the common binding site to ribosomes of macrolides, streptogramins and lincosamides shows that a mutation of the target causes cross-resistance to these three classes of antibiotics.

Future developments

Ketolides are 14-membered macrolides in which the cladinose moiety is replaced by a keto function.[25] These molecules remain active against organisms resistant through the inducible phenotype. Erythromycylamines modified at their cladinose moiety show activity against inducible resistant strains and also against strains resistant through the production of efflux pumps.

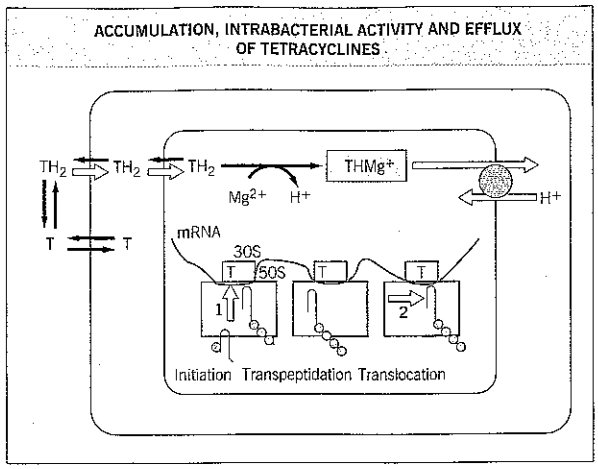


Fig. 1.9 Accumulation, intrabacterial activity and efflux of tetracyclines. Tetracyclines freely diffuse through the extracellular membrane of Gram-negative bacteria. Penetration inside bacteria is an energy-dependent process, depending on the pH (and Mg^{2+}) gradient between the extracellular medium of Gram-positive bacteria or the periplasmic medium of Gram-negative bacteria and the intracellular medium. Only the protonated form is highly diffusible, so that accumulation is favored by a lowering of the extracellular pH. Once inside the cytosol, the tetracycline molecule forms a nondiffusible complex with Mg^{2+}. This type of complex with a bivalent cation is also the substrate of the efflux pumps present in the membrane of resistant bacteria and acting as H^+ antiports (violet circle). The antibacterial action of the tetracyclines (T in the figure) is due to their binding to the 30S subunit of the ribosomes. In the pretranslocational state, tetracyclines inhibit the binding of aminoacyl-tRNA (arrow 1) to the A-site (green part of the ribosome). In the postranslocational state, tetracyclines protrude in the P-site (pink part of the ribosome) and inhibit the binding of the peptidyl-tRNA (arrow 2). Data from Geienmüller and Nierhaus, 1986[18] and Yamagushi *et al.*, 1990.[20]

LINCOSAMIDES

Chemical structure

Lincomycin and its 7-chloro-7-deoxy derivative, clindamycin, comprise a propylhygrinic acid linked to an aminosugar.

Mode of action

Lincosamides bind to the 50S ribosomal subunit and have a mode of action similar to that of macrolides. They inhibit early chain elongation by interfering with the transpeptidase reaction.

Resistance

The main mechanism of resistance to lincosamides is similar to that found in resistance to macrolides and streptogramins, and consists of alteration of the 50S subunit. Rare cases of enzymatic inactivation of the antibiotic have also been described for clindamycin (adenylation reaction).

Pharmacodynamics

Lincosamides are bacteriostatic, and are antagonists of macrolides and streptogramins, which bind at the same site on the ribosomes.

STREPTOGRAMINS

Chemical structure

Streptogramins are antibiotics that comprise a pair of synergistic constituents, namely a depsipeptide (group I) and a lactone macrocycle (group II).

Mode of action

Streptogramins bind to the 50S subunit of bacterial ribosomes and interfere with protein synthesis by a double mechanism that involves inhibition of both the incorporation of aminoacyl tRNA into the ribosomes and the translation of mRNA. The synergy between the two components may result from modification of the ribosome conformation caused by binding of the group I component, which exposes a fixation site for the group II component.[23]

Resistance

Resistance by mutation of the ribosomal target will also result in resistance to macrolides and lincosamides. Resistance to streptogramins alone is rare and occurs by enzymatic inactivation (involving an hydrolase and an acetylase).

Pharmacodynamics

Streptogramin constituents are highly synergistic and show a dose-dependent bactericidal activity if given together.[26] In addition, they increase the antibiotic activity of aminoglycosides and rifamycins.

Streptogramins also exhibit prolonged bacteriostasis, which consists of a delay of regrowth when the antibiotic concentration falls under its MIC. This could be interpreted as a consequence of the persistent binding of the drug to its target.

Future developments

These antibiotics are not widely used today. However, their potential use against bacteria that are resistant to other antibiotics (e.g. methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci) has reactivated research, and new products are now in development. In this regard, the combination of quinupristin and dalfopristin has recently received a favorable response from the Food and Drug Administration in the USA.[27]

CHLORAMPHENICOL AND THIAMPHENICOL

Chemical structure

Chloramphenicol and thiamphenicol are based on dichloroacetamide, bearing a diversely substituted phenyl group (see Chapter 7.11).

Bacterial targeting

Chloramphenicol acts principally by binding to the 50S subunit of the bacterial ribosomes. However, it can also interact with mitochondrial ribosomes of eukaryotic cells, which results in its toxicity.

Mode of action

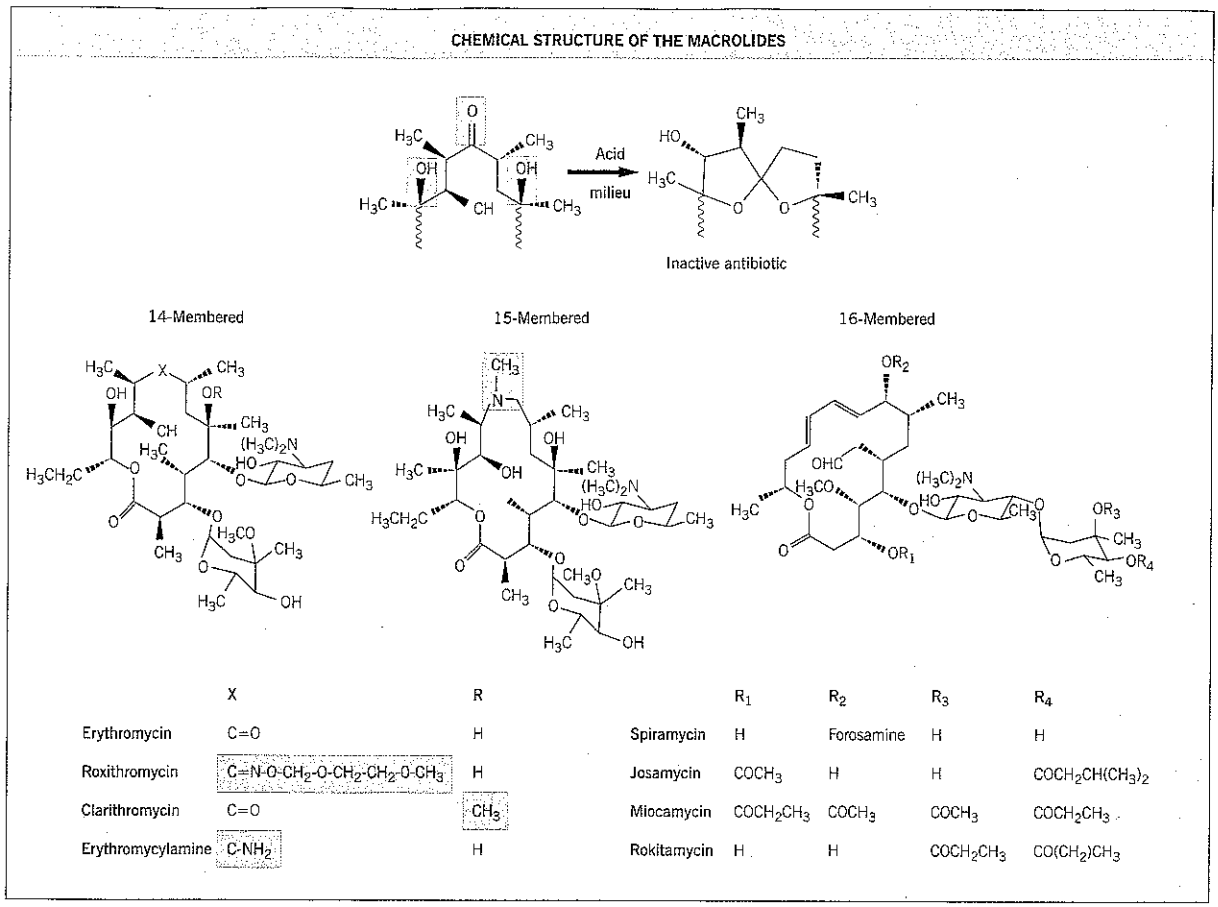
Chloramphenicol enters the bacteria by an energy-dependent process. Its antibiotic activity results from competitive inhibition of aminoacyl tRNA binding to the peptidyl transferase domain of the 50S subunit. This induces conformational changes to this part of the ribosomes, which slows or even inhibits (at high enough concentrations) the incorporation of aminoacyl tRNA and, therefore, the transpeptidase reaction.[28]

Resistance

Resistance to chloramphenicol derives mainly from the production of a specific acetyl transferase that inactivates the antibiotic.[29] The gene encoding the transferase is often located on plasmids that also confer resistance to other antibiotic classes. Another mechanism of resistance is reduced entry of the drug into the bacteria.

Pharmacodynamics

Chloramphenicol is bacteriostatic. It competes in binding to the ribosomes with macrolides and lincosamides, making its combination with these drugs useless.



	X	R
Erythromycin	C=O	H
Roxithromycin	C=N-O-CH_2-O-CH_2-CH_2-O-CH_3	H
Clarithromycin	C=O	CH_3
Erythromycylamine	C-NH_2	H

	R_1	R_2	R_3	R_4
Spiramycin	H	Forosamine	H	H
Josamycin	$COCH_3$	H	H	$COCH_2CH(CH_3)_2$
Miocamycin	$COCH_2CH_3$	$COCH_3$	$COCH_3$	$COCH_2CH_3$
Rokitamycin	H	H	$COCH_2CH_3$	$CO(CH_2)CH_3$

Fig. 1.10 Chemical structure of the macrolides. The upper panel shows the degradation of erythromycin in the gastric milieu (substituents responsible for the instability of the molecule are shown in gray). 16-Membered macrolides are intrinsically stable. The lower panel shows in gray characters the structural modifications conferring stability in acidic milieu to 14- and 15-membered macrolides.

DRUGS THAT AFFECT NUCLEIC ACIDS

FLUOROQUINOLONES

Chemical structure

Fluoroquinolones are totally synthetic products originally derived from nalidixic acid (see Chapter 7.9). All current compounds have a dual ring structure, with nitrogen at C1, a free carboxylate at C3 and a carbonyl at C4. A fluorine substituent at C6 greatly enhances activity, whereas substituents C7, C8 and N1 modulate the spectrum, pharmacokinetics and side effects of the drugs (Fig. 1.11).[30]

Bacterial targeting

Fluoroquinolones cross the outer membrane of Gram-negative bacteria via porins. Their affinity for the bacterial target is 1000 times greater than that of the corresponding eukaryotic enzyme, which ensures their specificity.

Mode of action

Fluoroquinolones inhibit the activity of topoisomerases, enzymes responsible for the supercoiling of DNA (DNA gyrase) and relaxation of supercoiled DNA (topoisomerase IV). Both enzymes have a similar mode of action, which implies:[31,32]

- binding of DNA to the enzyme,
- cleavage of the DNA,
- passage of the DNA segment through the DNA gate and
- resealing of the DNA break and release from the enzyme.

Gyrase and topoisomerase IV are tetramers made of two types of subunits, namely two GyrA or ParC that catalyze DNA cutting and resealing, and two GyrB or ParE responsible for the transduction and binding of adenosine triphosphate. The main target of fluoroquinolones is DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria.

Fluoroquinolones form a ternary complex with DNA and the enzyme (Fig. 1.12). This binding site for fluoroquinolones is formed during the gate-opening step of the double-strand DNA. Cooperatively, four fluoroquinolone molecules are fixed to ssDNA. Their stacking is favored by the presence of coplanar aromatic rings in their structure and by the tail-to-tail interactions between the substituents at N1. Interaction with DNA occurs by hydrogen bonds or via Mg^{2+} bridges established with carbonyl and carboxylate groups. Interaction with the enzyme is mediated by fluorine at C6 and substituents at C7. The binding of the fluoroquinolones stabilizes the cleavable complex

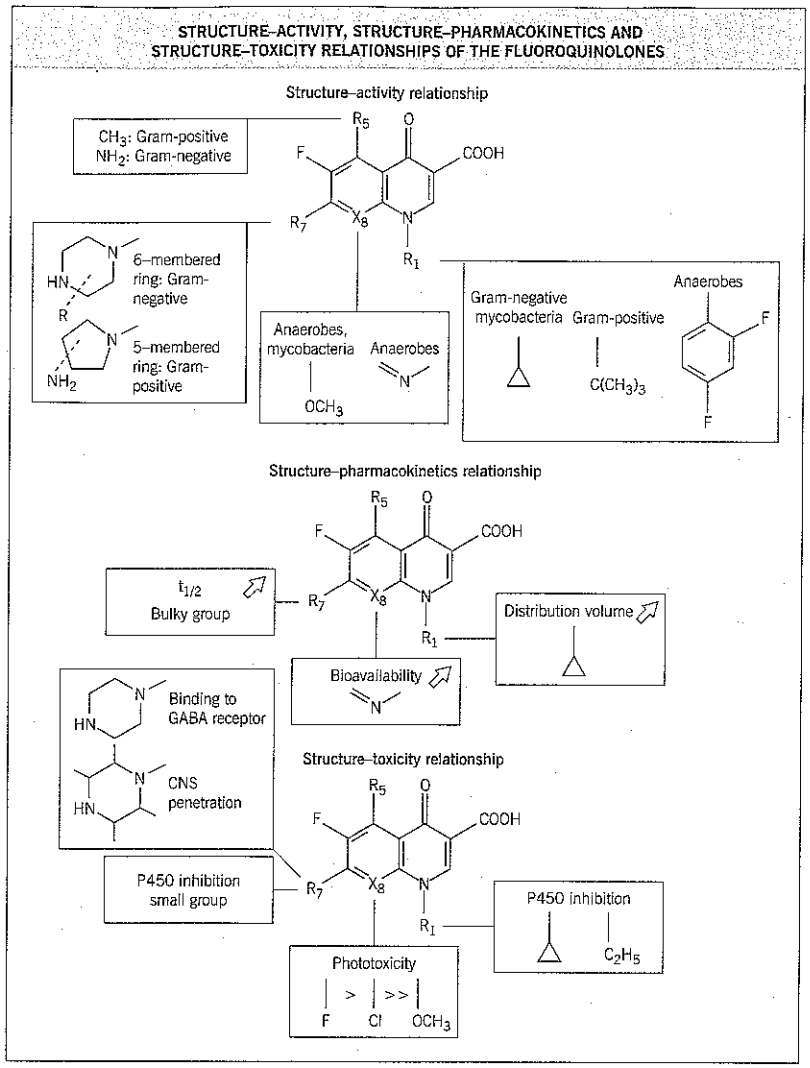


Fig. 1.11 Summarized view of the structure–activity, structure–pharmacokinetics and structure–toxicity relationships of the fluoroquinolones. These considerations form the basis of the rational development of the new molecules of this class, which have a very extended spectrum (including Gram-positive bacteria and anaerobes), a long half-life and minimal phototoxicty and metabolic interactions.

(formed by the cut DNA and the enzyme) and leads to the dissociation of the enzyme subunits. The latter action is observed only for potent molecules or at higher concentrations.

Quinolones have other effects on bacterial cells, such as induction of the DNA repair response, which involves three proteins (RecA, LexA and RecBCD). Induced RecA cleaves the repressor part of the SOS regulon (LexA), stimulating repair of damage caused by fluoroquinolones to DNA. Induced RecBCD binds to the chromosome at the double-strand break created by the ternary complex of topoisomerase–DNA–quinolone, and results in mutagenesis as well as increased cell survival in the presence of quinolones. This system therefore protects against the antibacterial activity of fluoroquinolones.[30]

Resistance

Resistance occurs mostly by mutation of the topoisomerases (reducing drug-binding ability), by porin impermeability, or by efflux. These mechanisms affect all fluoroquinolones and result in progressive slight increases in the MIC. Some fluoroquinolones may remain active against resistant strains based upon higher intrinsic activity, which is a structure-related property.

Pharmacodynamics

The mechanism described above requires RNA and protein synthesis as well as cell division for bactericidal action. The latter probably results from cutting of the DNA and the subsequent creation of a barrier for its transcription. Alternative mechanisms, however, confer bactericidal activity to certain molecules either in the absence of protein and RNA synthesis or without bacterial multiplication.

The activity of fluoroquinolones is largely concentration dependent and proportional to the amount of drug administered, which requires the use of large doses (pharmacodynamic studies have shown that effective doses often need to be considerably higher than originally thought and/or that breakpoints should be revised to lower values). Fluoroquinolones also show a postantibiotic effect, the duration of which varies according to the pathogen, drug concentration and period of exposure.

Future developments

New fluoroquinolones (among which trovafloxacin, moxifloxacin and grepafloxacin are now in clinical use) have been designed to cover Gram-positive organisms better, to maintain activity against Gram-negative organisms and to provide activity against anaerobes

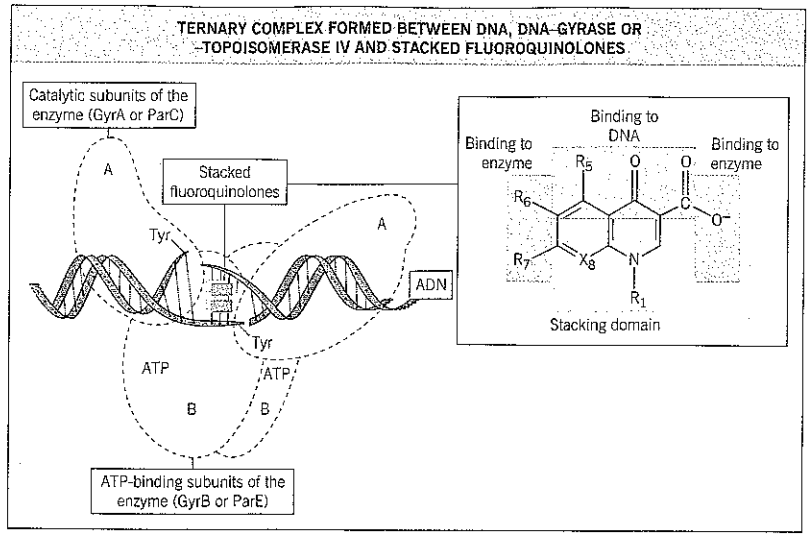


Fig. 1.12 Ternary complex formed between DNA, DNA–gyrase or –topoisomerase IV and stacked fluoroquinolones. Subunits A form covalent bonds via Tyr122 with the 5′ end of the DNA chain. The binding site for fluoroquinolones is located in the bubble formed during the local opening of the DNA molecule. The right panel of the figure shows the parts of the antibiotic molecules that interact with DNA, with the enzyme, or favoring the stacking of the fluoroquinolone molecules. Adapted from Shen *et al.*, 1989.[31]

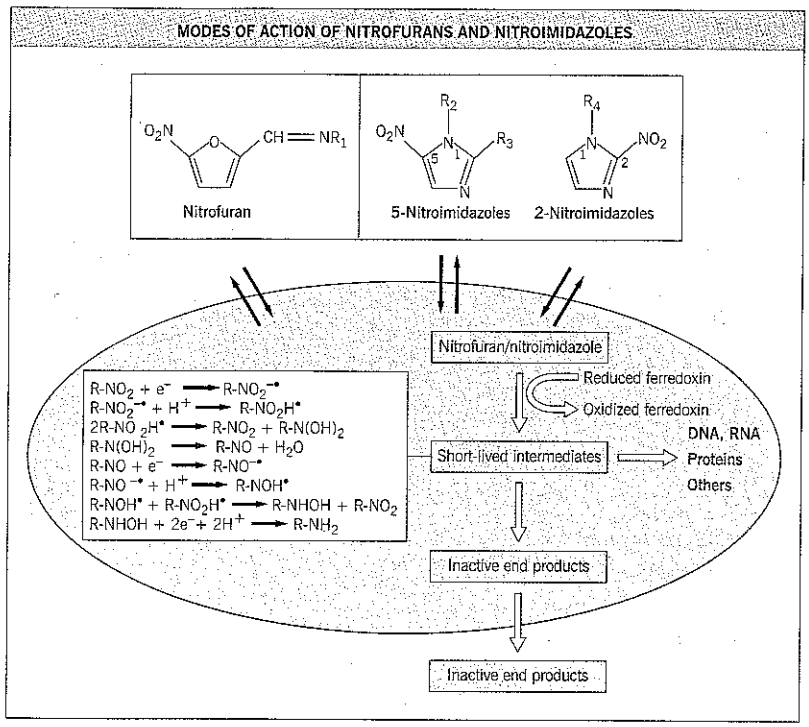


Fig. 1.13 Modes of action of nitrofurans and nitroimidazoles. The modes of action include passage through the cell membrane, reduction to highly reactive products, interaction with intracellular targets and release of inactive end products.

(trovafloxacin and to a lesser extent moxifloxacin).[33] They all contain a small hydrophobic substituent on N1 and a diamino, small ring substituent at C7. However, they remain ineffective against bacteria with MIC >1μg/ml (or about 0.3μg/ml based on pharmacodynamic considerations), if current recommended dosages are used.

NITROIMIDAZOLES AND NITROFURANS

Chemical structure

The nitroheterocyclic drugs include nitrofuran and nitroimidazole compounds (Fig. 1.13; see Chapter 7.12).

Mode and spectrum of action

The activity of nitroheterocyclic drugs requires activation of the nitrogroup attached to the imidazole or furan ring, which must undergo single- or two-electron enzymatic reduction in the bacteria.[34,35,36] Single-electron reduction of nitroaromatics is most frequently catalyzed by flavoenzyme dehydrogenase electrotransferases and bacterial oxygen-sensitive nitroreductases. Under aerobic conditions, the single-electron reduction of nitroaromatics to give their anion radicals results in their reoxidation by oxygen with formation of superoxide and other activated oxygen species that damage proteins, nucleic acids

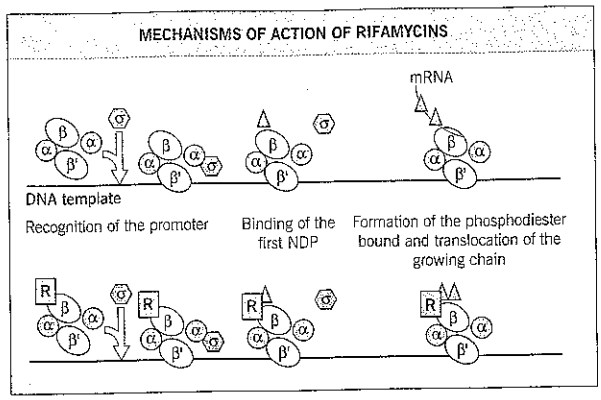


Fig. 1.14 Mechanisms of action of rifamycins. The synthesis of mRNA by RNA polymerase is shown in the upper panel and inhibition by rifamycins (R in the green squares) is shown in the lower panel. The RNA polymerase core is made of four subunits, among which the β′ subunit binds to the DNA template and the β subunit binds the ribonucleotide diphosphate (NDP; triangle). The α factor only participates to the initiation step by allowing for the recognition by the enzyme core of promoter sequences on the DNA template. Rifamycins bind to the β subunit. They do not interfere with the binding of the nucleotide diphosphate, but rather inhibit the transcription initiation either by impairing the formation of the first phosphodiester bond or the translocation reaction of the newly synthesized dinucleotide.

and lipids. Under hypoxic conditions, enzymes that transfer single electrons reduce nitroaromatics to amines or, less frequently, to hydroxylamines. The two-electron reduction of nitroaromatics to nitroso compounds and, subsequently, to hydroxylamines is catalyzed by bacterial oxygen-insensitive nitroreductases and mammalian DT-diaphorase NADPH:quinone reductase.

Although the nitro radicals generated by reduction of the parent drugs are similar for the nitroimidazoles and the nitrofurans, these drugs differ in their reduction potential, and therefore in their effects on bacteria and their spectrums of activity. Thus, the reduction of nitroimidazoles causes depletion in the intracellular stock of reduced coenzymes. Moreover, reduced forms of these antibiotics are highly reactive and may damage the DNA molecule. Reduced nitrofurans also inhibit the activity of enzymes involved in the degradation of glucose and pyruvate. In addition, they covalently bind to proteins and DNA by an alkylation reaction.

Future developments

The variety of substitutions that can be attached to the ring structures may allow a large amount of flexibility. The use of 2-nitroimidazole probes as radiosensitizers of hypoxic cells on a cell-to-cell basis and for noninvasive detection is currently the major interest in these drugs.

ANSAMYCINS

Chemical structure

Ansamycins, which are macrocyclic antibiotics, are lipophilic and therefore easily diffuse through membranes. They comprise two aromatic rings (containing a quinone) connected by a long chain (or 'ansa' – hence the name given to this class of antibiotics), which confers a rigid character to the whole molecule.

Mode of action

Ansamycins inhibit initiation of DNA transcription to mRNA and therefore the subsequent protein synthesis.[37] The RNA polymerase contains five subunits ($\alpha_2\beta\beta'\sigma$):

- α-subunits establish contact with transcription factors,
- β′-subunit is a basic polypeptide that binds DNA,
- β-subunit is an acidic polypeptide and is part of the active site, and
- σ-subunit initiates the transcription and then leaves the polymerase nucleus.

The core polymerase ($\alpha_2\beta\beta'$) therefore retains the ability to synthesize RNA but is defective in its ability to bind and initiate DNA transcription.

Inhibition by rifamycins follows binding of the antibiotic to the β-subunit of the RNA polymerase or, to a lesser extent, of the DNA–RNA complex. This binding is mediated by hydrophobic interactions between the aliphatic ansa chain and the β-subunit. The precise site of binding has been identified only partly, by studying mutants in RNA polymerase that have acquired resistance to rifampin (rifampicin). All the mutations that affect drug binding belong to three clusters of amino acids in the central domain of the β-subunit.

Inhibition of transcription caused by rifamycins is essentially noncompetitive. A model has been proposed in which rifamycins block the translocation event during transcription initiation, without hindering the synthesis of the first phosphodiester bridge between the two first nucleotide triphosphates of the mRNA molecule.[38] Specificity of action depends on the fact that ansamycins alter mammalian cell metabolism only at concentrations 10,000 times those necessary to cause bacterial cell death (Fig. 1.14).

Pharmacodynamics

Rifamycins are bactericidal, an effect that results either from the high stability of the complex formed between rifampin and the enzyme or from formation of superoxide ions on the quinone ring of the antibiotic molecule. As their action is to hinder bacteria multiplication, they are, at least *in vitro*, antagonists to antibiotics that require active bacterial growth to exert their activity (beta-lactams) or to other antibiotics that act on protein synthesis (macrolides and aminoglycosides). This antagonism is, however, not observed *in vivo*, because of the different distribution of these antibiotics (intracellular for rifamycins; extracellular for beta-lactams and aminoglycosides). Their postantibiotic effect is also long-lasting, again because of the irreversible nature of their binding. The efficient cell penetration of ansamycins gives them excellent activity against sensitive intracellular organisms.

Future developments

Benzoxazinorifamycins constitute a new group of semisynthetic molecules that show greatly enhanced activity against *Mycobacteria* spp., the main clinical target of this class of antibiotics.[39]

ANTITUBERCULOUS AGENTS

Other drugs that have specific activity against mycobacteria are discussed in Chapter 7.13.

ANTIMETABOLITES

SULFONAMIDES AND DIAMINOPYRIMIDINES

Prontosil (sulfamidochrysoidine) is a synthetic compound with antibacterial activity found by Domagk in 1932. In fact, this product was a prodrug that led to the development of the sulfonamides. With diaminopyrimidines, they inhibit the folate pathway in bacteria.

Chemical structure

Sulfonamides are derived from *p*-aminobenzenesulfonamide, which is a structural analog of *p*-aminobenzoic acid, a factor required by bacteria for folic acid synthesis. A free amino group at C4 and a sulfonamide group at C1 are required for antibacterial activity. Heterocyclic or aromatic rings substituting the sulfonamide enhance this activity by modifying absorption and gastrointestinal tolerance.

Diaminopyrimidines, such as trimethoprim and pyrimethamine, are pyrimidines substituted at C5 by an aromatic group. Pyrimethamine has an additional substituent at C6.

Mode of action

Sulfonamides inhibit tetrahydrofolic acid synthesis.[40,41] Briefly, this synthesis requires successive enzymatic reactions, among which are:

- formation of pteroic acid from *p*-aminobenzoic acid and dihydropteridin catalyzed by the dihydropteroate synthetase; and
- reduction of dihydrofolic acid into tetrahydrofolic acid (the active form of folic acid) catalyzed by the dihydrofolate reductase.

Sulfonamides act via a double mechanism. First, as analogs of *p*-aminobenzoic acid, they are competitive inhibitors of dihydropteroate synthetase. Second, they can also function as alternative substrates for the synthetase and become incorporated into a product with pteridine.

Diaminopyrimidines are specific inhibitors of bacterial dihydrofolate reductase,[40,41] and act as competitive inhibitors of this enzyme. Even though dihydrofolate reductase is present in bacteria as well as in eukaryotic cells, action selectivity occurs; this might be explained by the different conformation formed in the cavity of the bacterial enzyme compared with the conformation in the eukaryotic enzyme [radiographic cocrystallization data (trimethoprim–enzyme) suggest that trimethoprim in bacterial enzymes establishes more binding interactions than in the eukaryotic enzymes]. In addition, the NADPH cofactor may stabilize the enzyme–trimethoprim complex in the bacteria.

Resistance

For sulfonamides, resistance mainly occurs by hyperproduction of *p*-aminobenzoic acid or by reduction of the affinity of dihydrofolate reductase for the antibiotic, which causes resistance to the whole class. For diaminopyrimidines, resistance mostly occurs by enzyme mutations that prevent binding [a single point mutation (e.g. Phe98→Tyr) is sufficient to prevent any binding of trimethoprim to the enzyme, because of the loss of a critical hydrogen bound].[42]

Pharmacodynamics

Sulfonamides are only bacteriostatic, but in combination with diaminopyrimidines they have a definite antibacterial synergism and the combination is bactericidal.

CYCLIC POLYPEPTIDES (POLYMYXINS/COLISTINS)

Chemical structure

These are a collection of cyclic, branched polypeptides (molecular masses about 1000Da) containing both cationic and hydrophobic aminoacids. Some of these are of the D configuration or are non-DNA coded, which confers resistance to mammalian peptide-degrading enzymes (60 to 90 % of a parenterally-administered dosis is excreted intact in the urines). Polymyxins are obtained from *Bacillus polymyxa* and colistins from *Aerobacillus colistinus*. Only polymyxin B and colistin A (identical to polymyxin E) are used in clinical practice.

Mode of action

Because of their amphipathic character, polymyxins and colistins act as detergents and alter the permeability of the cytoplasmic membrane.[43] They, therefore, affect bacteria at all stages of development. They however cannot easily diffuse through the thick peptidoglycan layer of the Gram-postive bacteria. In contrast, they easily bind to the phospholipids of the outer membrane of the Gram-negative bacteria from where they reach the cytoplasmic membrane through polar as well as nonpolar channels. These properties explain both their strong and fast bactericidal activity through major perturbation of the inner membrane permeability properties (but recent findings have challenged tis mechanism) and their narrow spectrum essentially limited to Gram-negative organisms.

Resistance

Acquired resistance to polymyxins and colistins is chromosomal and results from a decreased permeability of the outer membrane secondary to changes in its biochemical composition. Bacteria with decreased sensitivity are indeed characterized by a decreased phospholipid/lipid ratio and a higher content in divalent cations (Ca^{++}; Mg^{++}). Protein H1 from *P. aeruginosa* (presently known as OprH) prevents binding of polymyxins and colistins to LPS and its overproduction has been correlated with lesser sensitivity [this change is, however, not sufficient *per se* and must be combined with other modifications of the membrane; two genes downstream to OprH (PhoP and PhoQ) coregulate OprH and polymyxin B resistance]. Although still exceptional, resistance to polymyxins and colistins has now been described in strains exhibiting multiple resistance to beta-lactams and aminoglycosides.[44]

Pharmacodynamics

Colistin A and polymyxin B show concentration-dependent activity but no or little post-antibiotic effect (rapid regrowth after the concentration falls below the MIC), justifying the administration of repeated daily doses.[45]

Non-antibiotic pharmacological and toxicological properties related to chemical structure

As membrane-disrupting and lipid-binding agents, polymyxins and colistins display a number of non-antibiotic effects, some of them potentially useful [inactivation of endotoxins (immobilized polymyxin B is currently used to remove endoxins from protein solutions), and synergy with serum bactericidal activities], but many others highly detrimental to the host [activation of the alternative pathway of complement, mastcell degranulation with histamine release, decreased production of cytokines (but increase in TNF-α release), increase in membrane conductance in epithelia, apoptosis].

Future developments

Because of the widespread emergence of resistance to other antimicrobials, polymixins are being re-evaluated for chronic, difficult-to-treat infections (e.g. pulmonary infections in cystic fibrosis patients) and new, potentially less-toxic derivatives are therefore being synthesised and evaluated.[46,47] The use of polymyxin B as anti-endotoxin agent is also investigated.[48]

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