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Infectious Diseases

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Chapter 130: Mechanisms of Action

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Chapter | **130** |

Mechanisms of action

ANTIBIOTICS THAT ACT ON THE CELL WALL

The basis of the bacterial cell wall is peptidoglycan, containing alternating residues of *N*-acetyl-glucosamine and muramic acid in β-1→4 linkage. The carboxyl groups of muramyl residues are substituted by short peptides (usually pentapeptides) terminated by a D-Asp-D-Ala-D-Ala sequence. Cell-wall active antibiotics act by inhibiting the activity of enzymes involved in the synthesis of the precursors or in the reticulation of peptidoglycan.

β-Lactams

The β-lactam nucleus is the basic building block of an exceptionally large class of antibiotics that all share a common mode of action but have quite distinct properties in terms of spectrum, pharmacokinetics and, to some extent, activity against resistant strains.

Chemical structure

All antibiotics in this class contain a cyclic amide called β-lactam. With the exception of the monobactams, this cycle is fused with a five- or six-membered cycle. According to the nature of the four-membered latter and/or of the heteroatom included, the following classes have been described (Fig. 130.1):

- penams – β-lactams with a five-membered ring containing a sulfur atom (penicillins);
- clavams – β-lactamase inhibitors that contain a five-membered ring with an oxygen as the heteroatom (e.g. clavulanic acid; sulfur analogues have also been reported);
- carbapenems – five-membered rings without heteroatom and with a double bond (e.g. thienamycin, imipenem);
- cephems – six-membered unsaturated rings with a sulfur atom (cephalosporins);
- oxacephems – the oxygen analogues of cephems (latamoxef);
- 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 β-lactams include thiacephems, dethiacephems, dethiacephams, heterocephems and cephams, as well as diverse bicyclic systems.

Mode of action

β-Lactams act primarily as inhibitors of transpeptidases (specialized acyl serine transferases), thereby impairing the synthesis of the cell wall (Fig. 130.2).[1] β-Lactams mimic the D-Ala-D-Ala sequence in that the distance between the carboxylate or the sulfonate (monobactams) and the cyclic amide is similar, and act as a false substrate for D-alanyl-D-alanyl transpeptidases. The carbonyl of the beta-lactam ring reacts with a serine residue of the transpeptidases (also called penicillin-binding proteins, PBPs) to give an inactive acyl enzyme ('suicide inhibition' by formation of a covalent bond; Fig. 130.3).[2] Transpeptidases, located in the periplasmic space, are directly accessible in Gram-positive bacteria but protected by the outer membrane in Gram-negative bacteria, which β-lactams must cross (mainly via porin channels).

The impairment of cell wall formation by β-lactams explains the inhibition of the growth of bacteria, but the bactericidal effect results from indirect mechanisms (mostly the activation of autolytic enzymes). β-Lactams are usually active against rapidly dividing bacteria only.

Resistance

Resistance to β-lactams occurs by three main different mechanisms.

- First, access to the PBPs in Gram-negative bacteria might be abolished by alteration of porin channels, which affects highly water-soluble β-lactams.
- Second, resistance can be conferred through modification of PBPs, in particular PBP2 which is essential for the 'shaping' of bacteria. The most typical example is found in methicillin-resistant *Staphylococcus aureus* (MRSA) that produces an altered protein (called PBP2′ or PBP2a) with a very low affinity for β-lactams. This makes the bacteria resistant to all conventional β-lactams. Recent research has shown that PBP2a is insensitive to β-lactams because the protein is in 'closed' conformation, preventing access of the drug to the reactive serine. This is circumvented if bacteria are exposed to acidic pH, which causes an opening of the enzymatic cavity.[3] This can also occur if β-lactams have a bulky hydrophobic substituent attached to the molecule in the vicinity of the carboxylate (see Fig. 130.4 for cephalosporins). These molecules typically show a loss of activity towards MRSA of only one to eight dilutions (corresponding to the energy needed to open the PBP2a) compared to fully susceptible *S. aureus* (in contrast with often more than 256-fold for conventional β-lactams). Two anti-MRSA β-lactam antibiotics (ceftobiprole and ceftaroline)[4] have been clinically developed. Penicillin-binding proteins in staphylococci or in other organisms (e.g. streptococci) can also show decreased affinity; in such cases resistance is more specific to some β-lactams but can also be only partial (decreased susceptibility).
- The third and for many years the most frequent mechanism of resistance is the production of hydrolyzing enzymes called β-lactamases.[5] The corresponding genes may be carried either on chromosomes (where their expression may be constitutive or inducible) or on plasmids, and their products are secreted out of the cell wall in Gram-positive bacteria and in the periplasmic space in Gram-negative bacteria. β-Lactamases are serine proteases that have a high affinity for β-lactams and cleave the amide bond. Although most β-lactamases open the β-lactam ring in the same way

Diversity of β-lactam antibiotics		
Structure	Group	Examples
	Penam	Penicillins
	Clavam	β-lactamase inhibitors (clavulanic acid)
	Carbapenem	(Thienamycin, imipenem)
	Penem	(Faropenem)
	Cephem	Cephalosporins
	Oxacephem	(Latamoxef)
	Monobactam	(Aztreonam)

Fig. 130.1 Diversity of β-lactam antibiotics: main ring structures names and representative antibiotics. The figure highlights the fact that all drugs share a common four-piece β-lactam ring and a carboxylate (or sulfonate) function at an appropriate, similar distance. (Clavams are weak antibiotics but efficient β-lactamase inhibitors.)

as transpeptidases, the hydrolysis rate is far quicker than in PBPs (see Fig. 130.3), resulting in the regeneration of the enzyme and the production of an irreversibly inactivated antibiotic. While PBPs turn slowly (one β-lactam per hour), the β-lactamases can turn on good substrates over 1000 times per second. X-ray data and genetic studies of β-lactamases and PBPs show a high level of structural homology, suggesting that both derive from a common ancestor.

A number of β-lactams have been made to resist to β-lactamases by appropriate steric hindrance or change in conformation (Fig. 130.5), giving rise to the large number of successive generations of so-called β-lactamase-resistant penicillins and cephalosporins. β-Lactamases, however, have an extraordinary plasticity and inevitably develop activity against all new derivatives at a rapid pace (Table 130.1).

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

Pharmacodynamics

β-Lactams are relatively slow-acting antibiotics that must be present at a concentration above the minimum inhibitory concentration (MIC) for as long as possible. Conversely, concentration above four to five times the MIC provides little gain in activity, so that frequent dosing is more appropriate than infrequent administration of large doses. Administration of β-lactams by continuous infusion is gaining increasing popularity,[6] but care must be taken regarding the stability of the drugs when exposed to room temperature for prolonged periods of time (carbapenems are unstable and cannot be used by continuous infusion; a 'prolonged' infusion time of 3–4 hours has been proposed for doripenem as part of the drug labeling). In general, β-lactams show only a moderate postantibiotic effect.

Future developments

Efforts continue to develop β-lactams active against MRSA in order to obtain compounds with increased intrinsic activity (applying the approach used with cephalosporins to penems). In parallel, efforts are being made to develop more effective β-lactamase inhibitors (especially for Gram-negative bacteria). Faropenem, an oral penem, was halted in its development in spite of excellent activity against pneumococci, largely because of fear of triggering resistance to the whole class of penems if used indiscriminately in the community.

Hybrid molecules combining a β-lactam part and an active antibiotic part from a different class (e.g. glycopeptides, fluoroquinolones) have also been described, with some potentially promising compounds (because of markedly improved activity) in development.

Glycopeptides and lipoglycopeptides

Chemical structure

Currently available glycopeptide antibiotics (vancomycin, teicoplanin) contain two sugars and an aglycone moiety made of a relatively highly conserved heptapeptide core, bearing two chloride substituents. 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, explaining why they are inactive against these organisms, but are also unable to penetrate inside bacteria, limiting them to an extracellular target. Lipoglycopeptides are semisynthetic derivatives characterized by the addition of a hydrophobic moiety, which confers additional properties to the drugs.

Mode of action

Glycopeptides inhibit the late stages of cell wall peptidoglycan synthesis (see Fig. 130.2) by binding 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 binding via their aglycone moiety.[7] The strength of this binding is greatly enhanced either by the dimerization of the glycopeptide molecules mediated by their sugars and the chloride atom on the aglycone (vancomycin) or by their anchoring in the membrane by a fatty acyl chain substituent (teicoplanin).[8] The subsequent steric hindrance around the pentapeptide termini blocks the reticulation of peptidoglycan by inhibiting the activity of transglycosylases (responsible for the fixation of a new disaccharide–pentapeptide subunit on the nascent peptidoglycan) and of transpeptidases (catalyzing the formation of interpeptide bridges).

Lipoglycopeptides (telavancin, oritavancin) add membrane-destabilization effects and stronger inhibition of the transglycosidase activities to the basic mode of action of the older molecules (resulting in the leakage of ions and other small molecules, causing rapid bacterial death). This is due to their membrane-anchoring properties (favored by the abundance of acidic phospholipids in bacterial membranes) and to dimerization of molecules[9] (Fig. 130.6) and, for oritavancin and enterococci, the possibility to bind an additional site in the petidoglycan.[9a] Dalbavancin (currently under development) also has a lipophilic side chain but does not appear to destabilize bacterial membranes, and results in exceptionally low MICs and a very prolonged half-life.
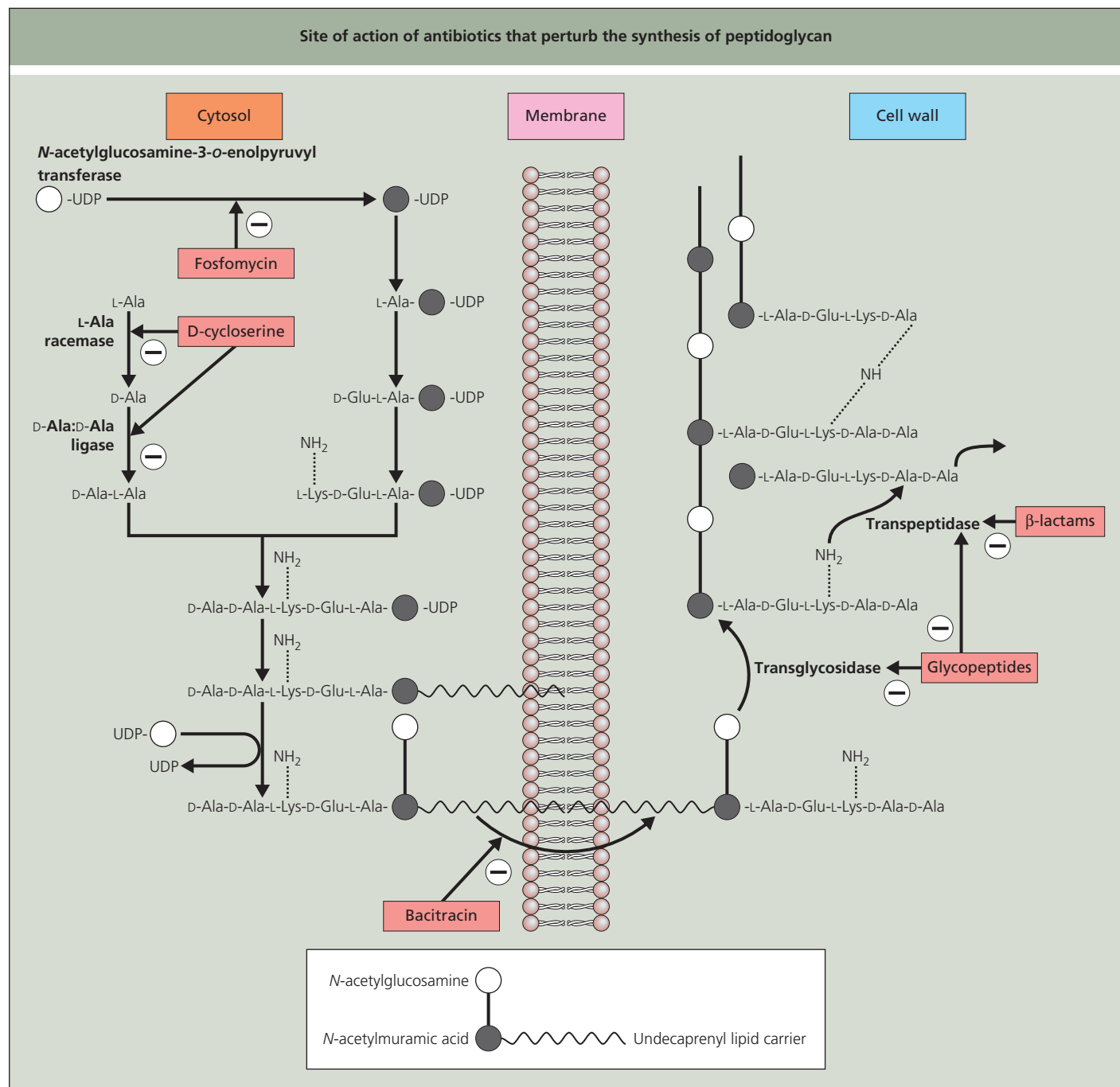
Fig. 130.2 Site of action of antibiotics that perturb the synthesis of peptidoglycan. The peptidoglycan unit is formed in the cytosol of bacteria by binding to uridine diphosphate (UDP) N-acetylmuramic acid of a short peptide (the nature of which differs between bacteria). This precursor is then attached 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 analogue of phosphoenolpyruvate, the substrate of the N-acetylglucosamine-3-O-enolpyruvyl transferase synthesizing N-acetylmuramic acid from N-acetylglucosamine and phosphoenolpyruvate; cycloserine is an analogue 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 β-lactams are analogs of D-Ala-D-Ala and suicide substrates for transpeptidases.

Resistance

Resistance to glycopeptides results from substituting a D-lactic acid or a D-serine in place of terminal D-Ala of the pentapeptide. While this does not prevent the action of the transpeptidase, it ruins the binding of the glycopeptides because of the loss of one crucial hydrogen bond.[7] This mode of resistance is most prevalent in enterococci, but rarely in *S. aureus*. In the latter, resistance is more commonly acquired as a result of

thickening of the cell wall associated with an increased abundance of free D-Ala-D-Ala termini. As a result, the MICs of the organisms increase modestly by about two- to eightfold, producing the so-called vancomycin- or glycopeptide-intermediate phenotype (VISA and GISA), eventually reaching a value of 4 mg/l or more at which clinically achievable serum concentrations of vancomycin can no longer inhibit bacterial growth (current CLSI and EUCAST breakpoints set a limit of 2 mg/l for susceptible strains). A further difficulty is the fact that the expression of
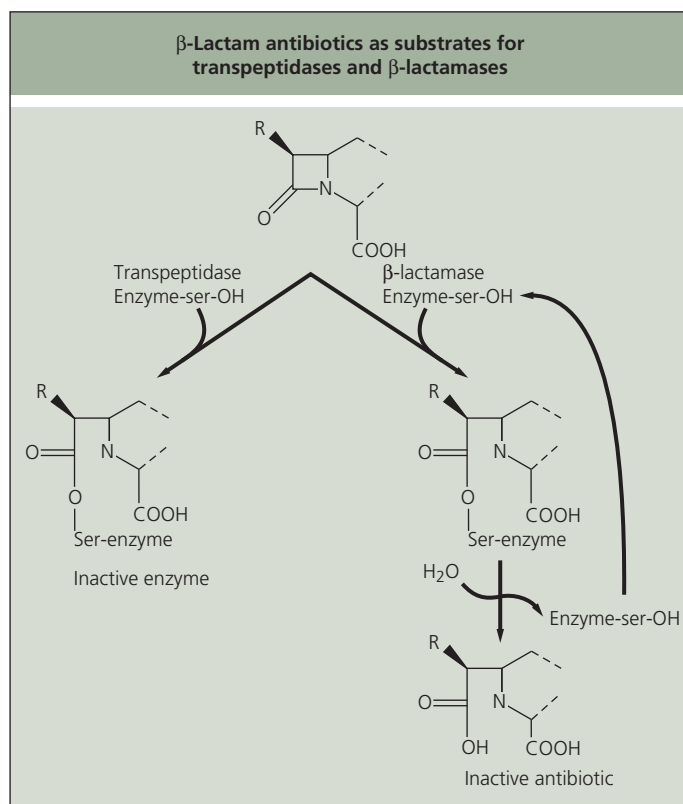
Fig. 130.3 β-Lactam antibiotics as substrates for transpeptidases and β-lactamases. The left part of the illustration shows how a β-lactam covalently binds to the transpeptidases. Hydrolysis of this acylated enzyme is very slow (one β-lactam per hour), making the enzyme inactive. The right part of the illustration shows that the same reaction occurs in the case of a β-lactamase. Hydrolysis of the acylated enzyme is, however, very rapid (1000 β-lactams per second), making the antibiotic inactive and regenerating the enzyme for a new cycle of hydrolysis.

this resistance is variable and may only affect a small proportion of a given inoculum, giving rise to a heteroresistance phenotype that makes detection by automated systems fairly unreliable.[10]

Lipoglycopeptides (telavancin, oritavancin) are partially immune to both resistance mechanisms thanks to their dual mode of action. It remains uncertain, however, whether keeping only the membrane-destabilizing effects (typically expressed only at higher drug concentrations than are needed to bind to D-Ala-D-Ala) will be sufficient to maintain clinically useful activity. Dalbavancin has variable activity against VISA strains and no useful activity against vancomycin-resistant organisms.
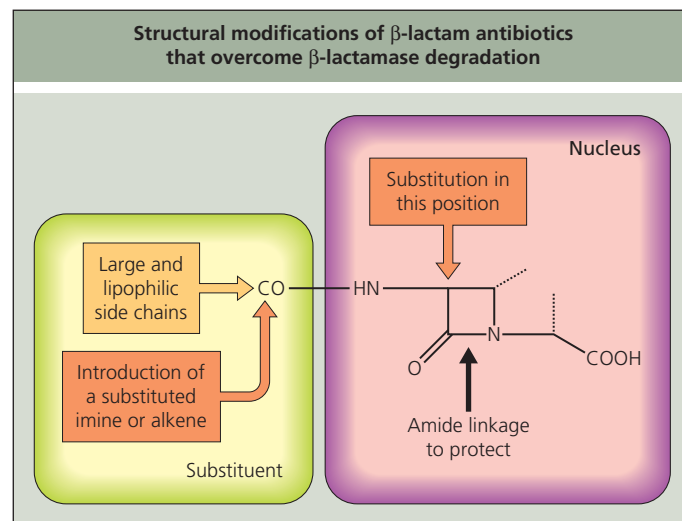

Fig. 130.5 Structural modifications of β-lactam antibiotics that overcome β-lactamase degradation. A first strategy, applied in penicillins, cephalosporins, oxacephems and monobactams consists of the introduction of a large side chain on the nucleus, possibly containing a substituted imine or alkene. A second strategy, applied in oxacephems and cefoxitin as well as in temocillin, consists of the introduction of a methoxy group on the β-lactam ring.
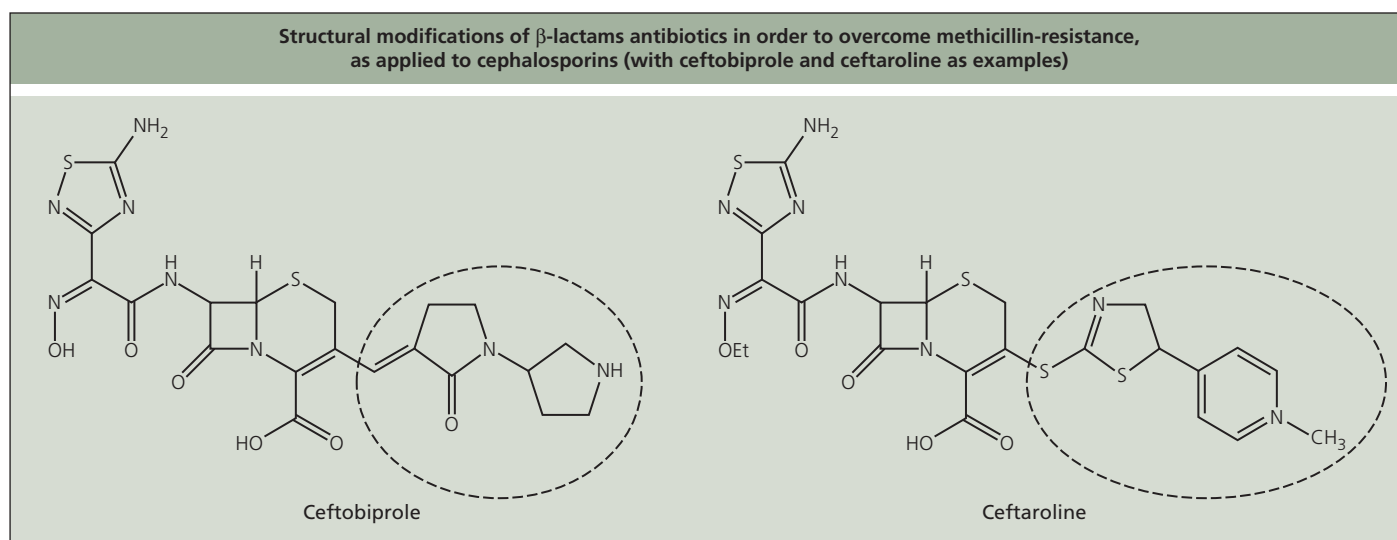

Fig. 130.4 Structural modifications of β-lactam antibiotics in order to overcome methicillin resistance, as applied to cephalosporins (with ceftobiprole and ceftaroline as examples). The bulky hydrophobic moieties (dotted-lined ellipse) added to the molecules forces a conformational change in PBP2a resulting in the opening of the active site and allowing acylation (inactivation) by the antibiotic. Although activity is largely restored towards methicillin-resistant organisms, MICs remain still typically one to four dilutions higher than for susceptible ones. The increase in lipophilicity also makes it necessary to administer the molecules as prodrugs – medocaril for ceftobiprole and fosamyl for ceftaroline (not shown).

Pharmacodynamics

Conventional glycopeptide antibiotics exhibit slow bactericidal activity, which is concentration independent. Because of their half-life (about 8 hours for vancomycin and much longer for teicoplanin), conventional glycopeptides display a 24-hour area under the serum concentration–time curve/MIC (AUC_{24h}/MIC) rather than a time above MIC phamacokinetic/pharmacodynamic pattern of activity.[11] This means that their mode of administration – whether discontinuous (q12h for vancomycin) or continuous – is similar. There is an increasing tendency to use vancomycin by continuous infusion (favored by its excellent aqueous stability) and to rely on trough rather than peak levels for optimum therapy. This contrasts with lipoglycopeptides, for which C_{max}/MIC ratios are probably more important. Lipoglycopeptides accumulate in macrophages and act on phagocytosed *S. aureus*.

Glycopeptides exhibit a synergistic effect with aminoglycosides, probably by facilitating the penetration of the latter drugs in bacteria.

Future developments

Talavancin has approved in 2009 for clinical use in the US, while oritavancin is still under development due to safety issues. Future developments should be geared towards clinical demonstration of superiority of these highly bactericidal molecules in comparison with vancomycin for 'difficult' indications.

Other agents acting on the cell membrane and cell wall synthesis

Daptomycin is a cyclic peptide flanked by an oxodecyl side chain conferring a strong amphiphilic character to the molecule (molecules in this group are often referred to as lipopeptides or peptolides; this erroneously suggests high lipophilicity, which is not true for daptomycin since the octanol–water partition is actually quite negative).

Table 130.1 Functional classification of β-lactamases

Group	Molecular class	Preferred substrates	Active β-lactams	Typical examples
Group 1: serine cephalosporinases not inhibited by clavulanic acid	C	Cephalosporins I, II, and III (>> cephalosporins IV, monobactams, penicillins)	Carbapenems Temocillin (cephalosporins III and IV; variable upon level of expression)	AmpC from Gram-negatives; variable upon the species
Group 2: serine β-lactamases				
2a: penicillinases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbenicillin >> oxacillins)	Amoxicillin + clavulanic acid Cephalosporins Carbapenems	Penicillinases from Gram-positives
2b: broad-spectrum β-lactamases inhibited by clavulanic acid	A	Penicillins (penicillin, ampicillin >> carbenicillin >> oxacillins) Cephalosporins I and II	Cephalosporins III and IV Monobactams* Carbapenems Amoxicillin + clavulanic acid	TEM-1,TEM-2, SHV-1 from Enterobacteriaceae, *Haemophilus* spp., *Neisseria gonorrhoeae*
2be: extended spectrum β-lactamases inhibited by clavulanic acid (ESBL)	A	Penicillins Cephalosporins I II III (IV) Monobactams	Carbapenems Temocillin	TEM-3 to -29, 42, 43, 46–49, 52–58, 60, 61, 63, 65, 66, 72, 92 from Enterobacteriaceae SHV-2 to -9, 11–14, 18–22, 24 from *Klebsiella* spp. CTX-M-1 to CTX-M-54 (five phylogenetic groups) in Enterobacteriaceae K1-OXY from *Klebsiella oxytoca*
2br: broad spectrum β-lactamases with reduced binding to clavulanic acid	A	Penicillins	Most cephalosporins Monobactams* Carbapenems	TEM-30 to -41 (= IRT-1 to IRT-12), 44, 45, 50, 51, 59, 68, 73, 74, 76–79, 81–84 from *Escherichia coli* SHV-10 from *Klebsiella* spp.
2c: carbenicillin-hydrolyzing β-lactamases 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 β-lactamases generally inhibited by clavulanic acid	D	Penicillins Cloxacillin Cephalosporins I and II	Carbapenems Cephalosporin III Monobactams* Piperacillin + tazobactam	OXA-1 to OXA-4 in Enterobacteriaceae OXA-2, OXA-10 (PSE-2) in *Pseudomonas aeruginosa* (pencillins, cefpirome, cefepime >> cephalosporins III) OXA-11 to -19, 28, 32, 45 are ESBLs in *P. aeruginosa* (R to Ceph 3, Ceph 4 and aztreonam) OXA-23, -24, -58 are carbapenemases in *Acinetobacter baumannii*

Table 130.1 Functional classification of β-lactamases—cont'd

Group	Molecular class	Preferred substrates	Active β-lactams	Typical examples
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-hydrolyzing-nonmetallo β-lactamases	B	Most β-lactams, including carbapenems (low or high resistance level depending on enzyme, species and genetic environment)	Carbapenems Monobactams and β-lactam inhibitors (variable activity depending on type of enzyme, bacterial host and genetic environment)	SME-1 to -3 from *Serratia* spp. IMI-1/2 and NMC-A from *Enterobacter cloacae* KPC-1 to -4 from *Klebsiella* spp. other Enterobacteriaceae and *Pseudomonas* GES-1 to -11 in Enterobacteriaceae, *P. aeruginosa* and *A. baumannii*
Group 3: metallo-β-lactamases inhibited by EDTA	B	Most β-lactams, including carbapenems	Monobactams*‡	L-1, XM-A from *Stenotrophomonas maltophilia* CcrA from *B. fragilis* A2h, CphA from *Aeromonas hydrophila* IMP-1 to -23, VIM-1 to -18 in *Pseudomonas*, other Gram-negative nonfermenters and Enterobacteriaceae SPM-1, GIM-1, SIM-1, DIN-1 in *P. aeruginosa* and *A. baumannii*
Group 4: penicillinases not inhibited by clavulanic acid		Penicillins, including carbenicillin and oxacillins	Monobactams*‡ and generally carbapenems	SAR-2 from *B. 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.
EDTA, ethylenediaminetetraacetic acid.
Compiled from [62, 63, 64, 65]. The number of enzymes as well as their spectrum of activity is continually evolving.

Daptomycin is used against vancomycin-resistant enterococci for staphylococcal infections. It has a novel mode of action, in that the molecule binds to Ca^{2+} to form an oligomeric assembly with the lipid tails pointing inwards. The loose micelles serve to deliver daptomycin to the bacterial membrane in a 'detergent-like' form, causing leakage of cytosolic contents and a rapid bactericidal effect (Fig. 130.7).[12] Daptomycin is only active against Gram-positive bacteria since it cannot cross the outer membrane of Gram-negative organisms. Daptomycin shows preferential interaction with the phospholipid, phosphatidylglycerol, which is abundant in prokaryotic cell membranes and largely absent from eukaryotic cell membranes, except in lung surfactant where it forms aggregates, thereby explaining the failure of daptomycin in treating pulmonary infections.

Resistance to daptomycin has already been described, resulting from mutations in genes that encode enzymes involved in the synthesis of phosphatidylglycerol. *Staphylococcus aureus* with a VISA phenotype (see glycopeptides section) are less susceptible to daptomycin due to impaired access through the thickened cell wall.[9]

Daptomycin activity is concentration dependent, whereas its toxicity (mainly for skeletal muscle) is more related to the frequency of exposure. As a result, daptomycin should be administered once daily. The original dose during development was 4 mg/kg. Whether this will be sufficient for difficult-to-treat staphylococcal infections and can be increased without eliciting unacceptable toxic reactions is presently investigated.

D-Cycloserine is a broad-spectrum antibiotic which has structural similarities to D-Ala (see Fig. 130.2; Fig. 130.8) and inhibits the conversion of L-Ala into D-Ala (catalyzed by a racemase) and the dimerization of D-Ala (catalyzed by the D-Ala:D-Ala ligase).[13]

Fosfomycin, which bears structural similarities to phospho-*enol*-pyruvate, inhibits very early stage synthesis of peptidoglycan by impairing the formation of uridine diphosphate (UDP)-*N*-acetyl-glucosamine-*enol*-pyruvate, a precursor of UDP-*N*-acetylmuramic acid (see Figs 130.2, 130.8).[14]

Bacitracin is a polypeptide of complex structure. It acts as an inhibitor of peptidoglycan synthesis at the level of translocation of the precursor across the bacterial membrane (see Fig. 130.2).[15]

ANTIBIOTICS THAT ACT ON PROTEIN SYNTHESIS

Bacterial ribosomes comprise:
- a 30S subunit that binds mRNA and initiates protein synthesis; and
- a 50S subunit that binds aminoacyl tRNA, catalyzes peptide bond formation and controls the elongation process.
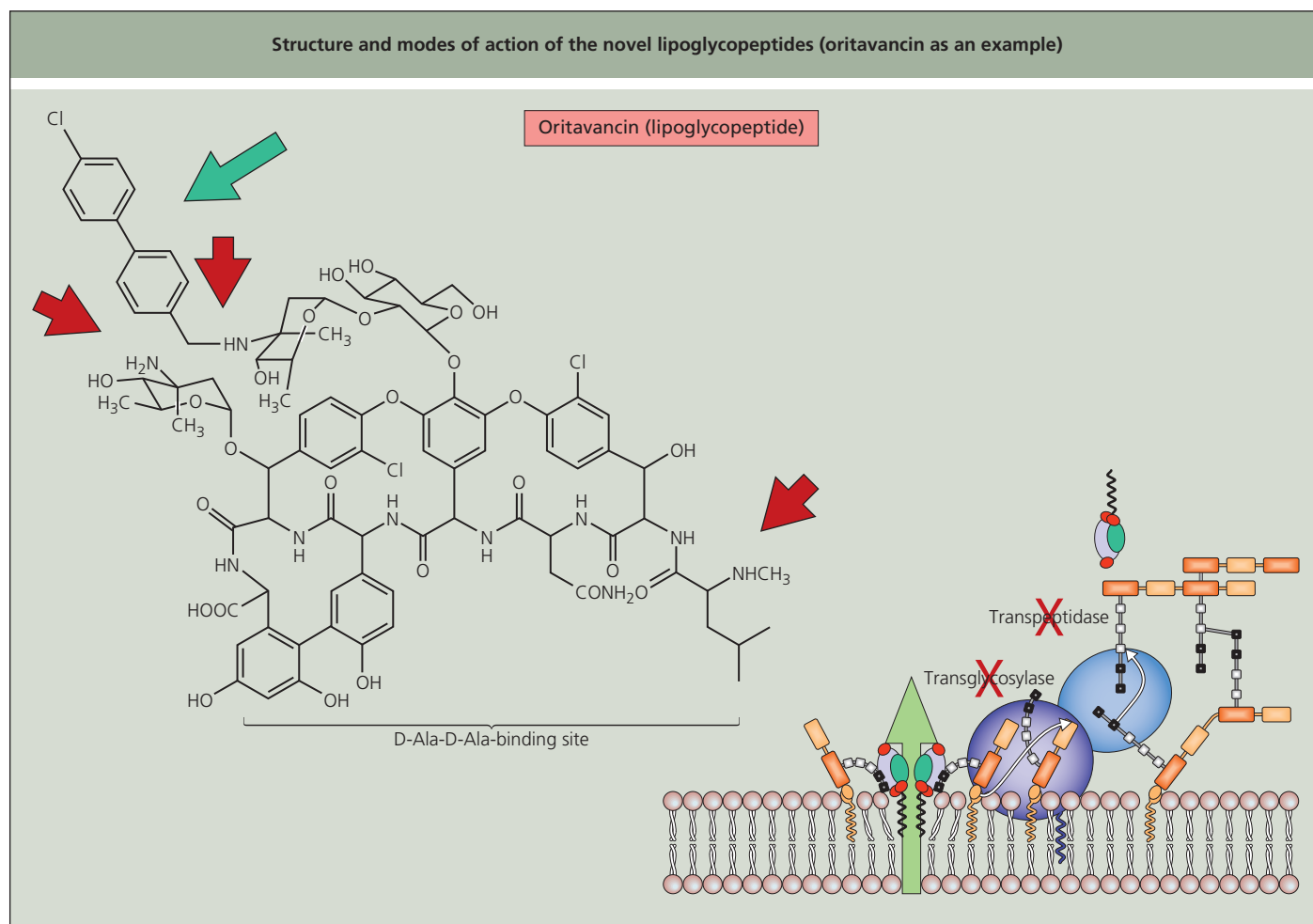
Fig. 130.6 Structure and proposed modes of action of the novel lipoglycopeptides (oritavancin as an example). Inset: the drugs are characterized by the presence of a bulky hydrophobic moiety (chorodiphenyl in this example; dark green arrow) and either additional amino groups (red arrows) or other polar groups (phosphonate for telavancin; not shown), resulting in a global amphiphilic character for the whole molecule. Note that the D-Ala-D-Ala binding site of the molecule remains unchanged. Main figure: the molecule interacts with the D-Ala-D-Ala termini of the pentapeptide (closed squares) as vancomycin, inhibiting both the transpeptidase and the transglycosidase activities, but also inserts itself into the membrane through its lipophilic side chain (this is favored, in the case of oritavancin, by its strong capacity to dimerize). As a result, lipoglycopeptides induce membrane leakage and thereby display a marked, concentration-dependent bactericidal effect. This second mode of action is exerted even if bacteria display a D-Ala-D-Lac or D-Ala-D-Ser in place of the D-Ala-D-Ala moiety, in as in vancomycin-resistant organisms. Specificity towards bacterial membranes probably stems from the fact that membrane-anchoring is enhanced by the presence of phospatidylglycerol, an acidic phospholipid abundant in bacterial but not in eukaryotic cell membranes. Adapted from Van Bambeke et al.[9]

The main sites identified in the 50S unit 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, discovered in 1944, has a limited spectrum of activity. Other aminoglycosides with a broader spectrum (kanamycins or gentamicins) have subsequently been obtained from natural sources. In the 1970s, the development of netilmicin and amikacin demonstrated the possibility of obtaining compounds active against strains resistant to earlier aminoglycosides.

Aminoglycosides comprise several aminohexoses joined by glycosidic linkages to a dibasic cyclitol.[16] The latter is streptidine in streptomycin and its derivatives, fortamine in the fortimicin series and 2-deoxystreptamine in most clinically used aminoglycosides.

This 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 for the kanamycin family; gentamicin C_1, C_{1a}, C_2, sisomicin, netilmicin and isepamicin for the gentamicin family; Fig. 130.9). All compounds are positively charged at physiologic pH.

Bacterial targeting

Aminoglycosides selectively inhibit bacterial protein synthesis by binding to the 30S ribosomal subunit. However, molecules displaying a hydroxyl function in C6′ in place of an amino function (G-418, also known as geneticin) can also affect protein synthesis of eukaryotes.

Mode of action

Although the highly polar nature of aminoglycosides prevents diffusion through membranes, they do cross the outer membrane of
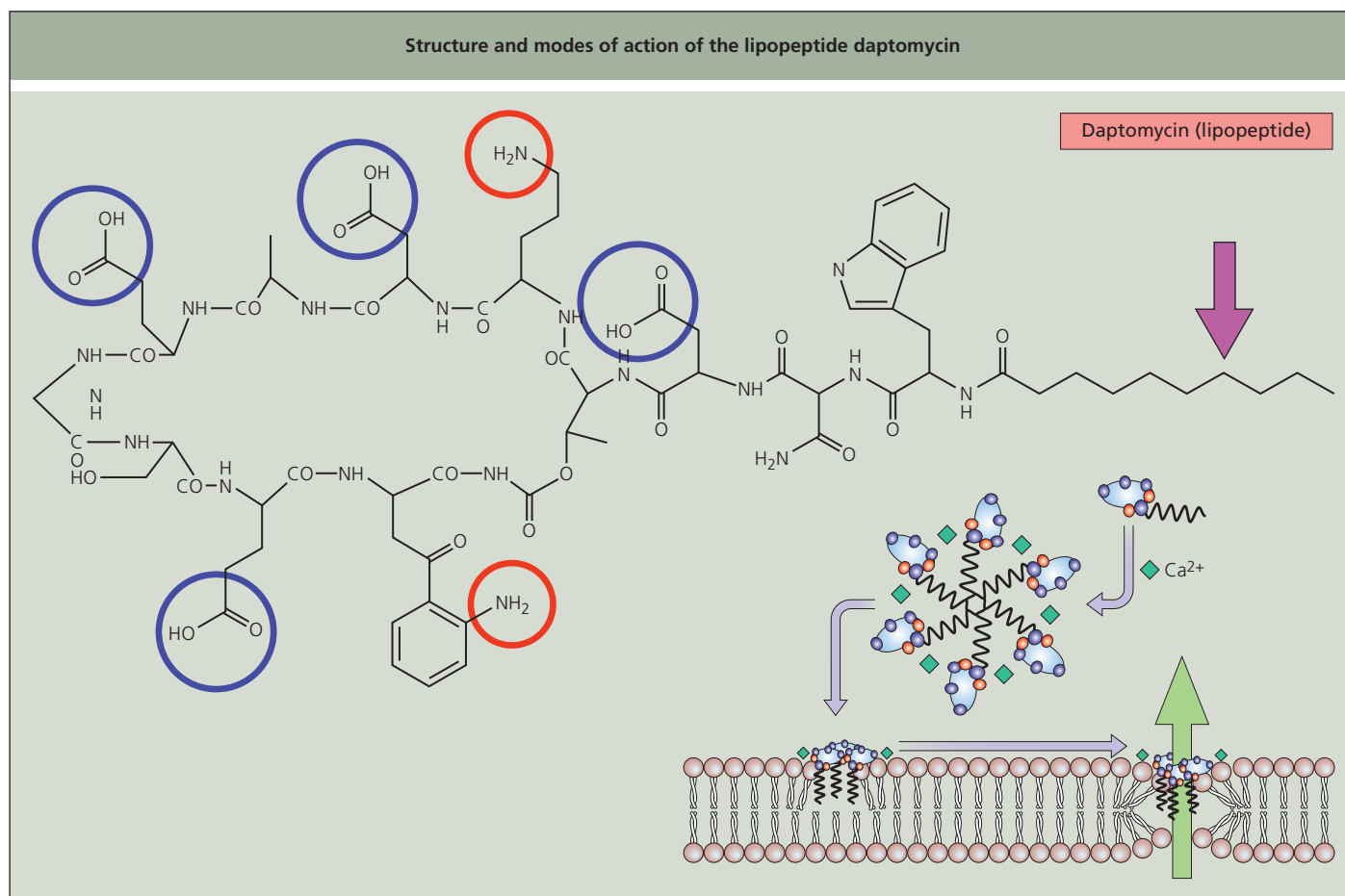
Fig. 130.7 Structure and mode of action of the lipopeptide daptomycin. The drug is a cyclic, polar depsipeptide (the ionizable residues are circled) flanked with a lipophilic oxodecyl side chain (purple arrow) conferring to the molecule a marked amphiphilic character. In the presence of Ca²⁺, daptomycin forms loose micelles that serve as a delivery system to the bacterial membrane where the drug lipophilic side chain can then interact with the fatty acid chains of the phospholipids causing permeabilization and rapid bacterial death. As for lipoglycopeptides, specificity towards bacterial membranes stems from the fact that daptomycin–membrane interactions are favored by the presence of phosphatidylglycerol, an acidic phospholipid abundant in bacterial but not in eukaryotic cell membranes (it is, however, present in lung surfactant, causing daptomycin inactivation in this environment and explaining clinical failures in pulmonary infections). Modified from Van Bambeke *et al.*[9]
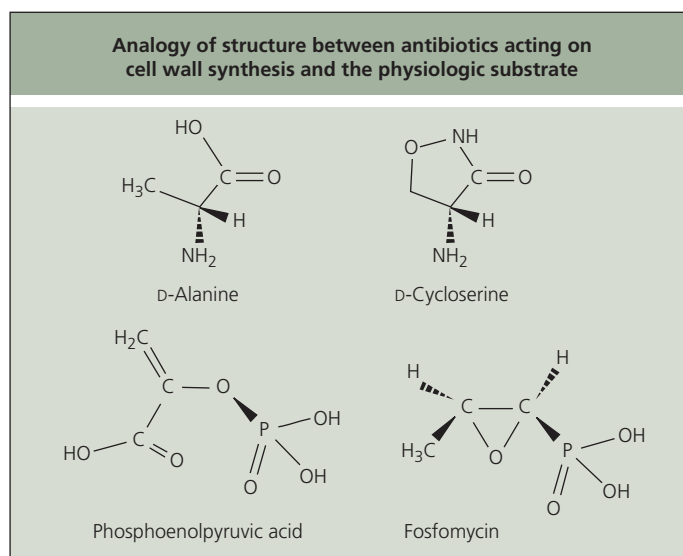
Fig. 130.8 Analogy of structure between cycloserine and fosfomycin and the corresponding physiologic substrates involved in peptidoglycan synthesis.

Gram-negative bacteria through a non-energy-dependent process involving drug-induced disruption of Mg²⁺ bridges between adjacent lipopolysaccharide molecules. Their transport across the cytoplasmic (inner) membrane of both Gram-positive and Gram-negative bacteria is dependent upon electron transport and is therefore termed energy-dependent phase I (EDP-I). Being related to the capacity of the bacteria to maintain a transmembrane electrical potential, this transport is impaired in anaerobic environments, at low external pH or in high osmolar culture media, which explains the corresponding reductions of antibacterial activity.

Once in the bacterial cytosol, aminoglycosides bind largely to the aminoacyl site of the 30S subunit of ribosomes and, to a lesser extent, to specific sites of the 50S subunit, again through an energy-dependent process (energy-dependent phase II, EDP-II), disturbing the elongation of the nascent peptide. Their mechanism of action is complex, involving inhibition of the transfer of peptidyl tRNA from the A-site to the P-site and impairment of the proofreading process that controls translational accuracy.[17] The aberrant proteins may be inserted into the cell membrane, leading to altered permeability and further increasing aminoglycoside transport, which contributes to and explains the highly bactericidal, concentration-dependent activity of aminoglycosides. (Binding of aminoglycosides to RNA has also been related to antiviral effects and impairment of eukaryotic protein synthesis, which, however, are not observed under conditions of clinical usage of presently developed aminoglycosides.)
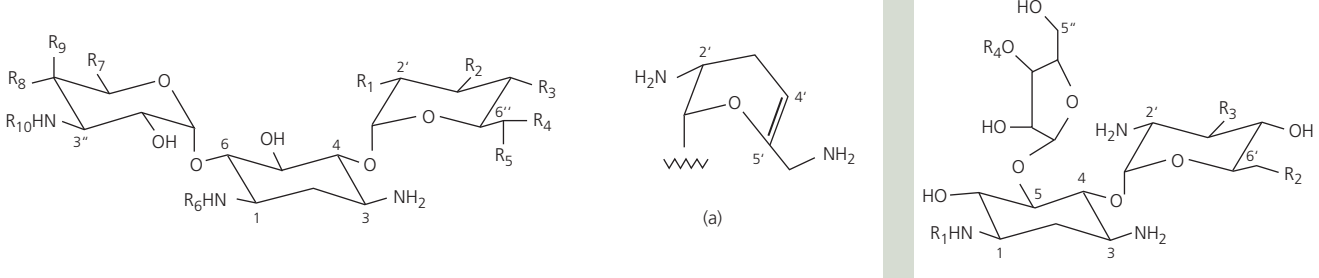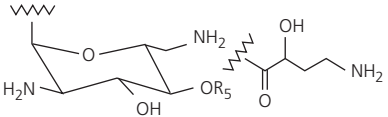
Structural formulae of the 2-deoxystreptamine-containing aminoglycosides

	Aminoglycoside	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10
Kanamycins	Kanamycin A	OH	OH	OH	H	NH2	H	CH2OH	OH	H	H
	Kanamycin B	NH2	OH	OH	H	NH2	H	CH2OH	OH	H	H
	Kanamycin C	NH2	OH	OH	H	OH	H	CH2OH	OH	H	H
	Amikacin	OH	OH	OH	H	NH2	COR'	CH2OH	OH	H	H
	Tobramycin	NH2	H	OH	H	NH2	H	CH2OH	OH	H	H
	Dibekacin	NH2	H	H	H	NH2	H	CH2OH	OH	H	H
	Arbekacin*	NH2	H	H	H	NH2	COR'	CH2OH	OH	H	H
Gentamicins	Gentamicin C1†	NH2	H	H	CH3	NHCH3	H	H	CH3	OH	CH3
	Gentamicin C1a†	NH2	H	H	H	NH2	H	H	CH3	OH	CH3
	Gentamicin C2†	NH2	H	H	CH3	NH2	H	H	CH3	OH	CH3
	Gentamicin C2b†	NH2	H	H	H	NHCH3	H	H	CH3	OH	CH3
	Gentamicin B†	OH	OH	OH	H	NH2	H	H	CH3	OH	CH3
	Isepamicin†	OH	OH	OH	H	NH2	COR	H	CH3	OH	CH3
	Sisomicin	–	–	–	–	–	H	H	CH3	OH	CH3
	Netilmicin	–	–	–	–	–	R''	H	CH3	OH	CH3

R = CHOHCH2NH2; R' = CHOH(CH2)2NH2; R'' = CH2CH3   (a) = primed sugar for sisomicin and netilmicin

*In Japan

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

4, 5-Disubstituted deoxystreptamine

Aminoglycoside	R1	R2	R3	R4	R5
Neomycin B	H	NH2	OH	X	H
Paromomycin I	H	OH	OH	X	H
Lividomycin A	H	OH	H	X	Mannose
Ribostamycin	H	NH2	OH	H	
Butirosin B	Y	NH2	OH	H	

Fig. 130.9 Structural formulae of the 2-deoxystreptamine-containing aminoglycosides. The numbering of the atoms shown is as in Mingeot-Leclercq *et al.*[16] with the primed numbers (') being ascribed to the sugar attached to C4 of the 2-deoxystreptamine (as this C is of the R configuration) and the doubly primed numbers (") being ascribed to the sugar attached to either the C6 (S configuration) for the 4,6-disubstituted 2-deoxystreptamine or the C5 (R configuration) for the 4,5-disubstituted 2-deoxystreptamine. (i) Eventually no molecule is shown in bold; (ii) the choice of one or another molecule is highly variable from one country to another (for non truly scientific reasons).

Resistance

Resistance occurs mainly from the production of aminoglycoside-modifying enzymes (Fig. 130.10). The semisynthetic derivatives (e.g. netilmicin, amikacin, isepamicin) were specifically designed to protect against the principal enzymes. However, multienzyme-producing bacteria have become increasingly common, causing multidrug resistance.[16] These enzymes may have physiologic functions against natural substrates and only target aminoglycosides opportunistically. However, point mutations and selection have quickly increased specificity and efficacy.[18]

A second mechanism of resistance causes membrane imperme-abilization, the underlying mechanism of which is mainly active drug efflux, with at least five distinct systems described in bacteria of medical interest such as *Escherichia coli* and *Pseudomonas aeruginosa*.[19] Because efflux proteins seem to capture their substrate from the inner, hydrophobic core of membranes and since aminoglycosides are polar, it is believed that it is actually a combination of aminoglycosides and phospholipids that serves as a substrate for efflux.

A third mechanism involves post-transcriptional methylation of 16S rRNA occurring in Enterobacteriaceae and nonfermenters. This affects all currently used 2-deoxystreptamine-containing aminoglycosides, with at least six distinct genes reported worldwide.[20]

Pharmacodynamics

Aminoglycosides demonstrate rapid, concentration-dependent killing as well as an important postantibiotic effect, probably due to a largely irreversible binding to the ribosomes. Once-a-day regimens provide the optimal mode of administration, producing high peak serum concentrations. Simultaneously, toxicity (renal and auditory) is delayed as uptake of the drug into the target tissues is saturable. As a result, a once-daily schedule is preferred.

Aminoglycosides are synergistic with antibiotics acting on cell wall synthesis by facilitating bacterial penetration of the aminoglycoside. In contrast, their activity is antagonized by bacteriostatic agents such as chloramphenicol and tetracyclines, probably by inhibition of their energy-dependent uptake and interference with the movement of the ribosome along mRNA.

Future developments

Efforts have been directed at:
- increasing the binding affinity while retaining binding selectivity; and
- developing derivatives resistant to aminoglycoside-inactivating enzymes, with one novel molecule (ACHN-490 [6' (hydroxylethyl)-1-(haba)-sisomicin]) currently in clinical development.
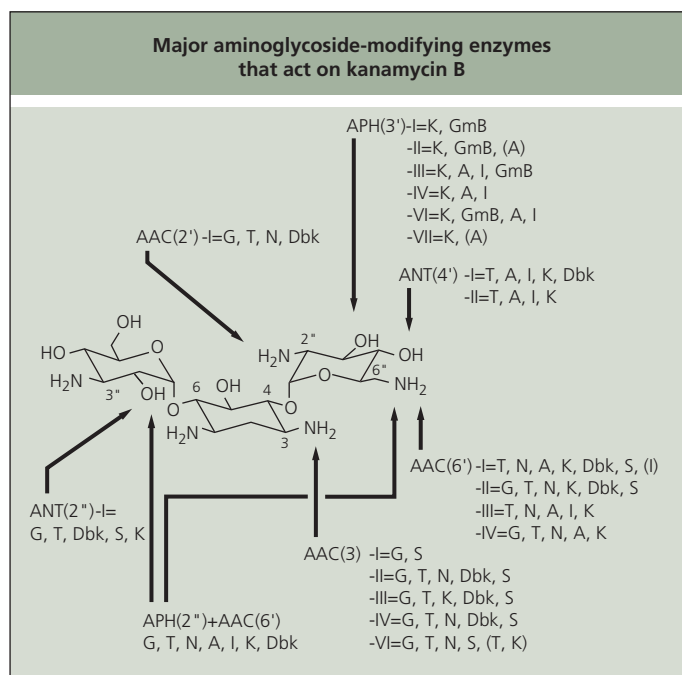
Fig. 130.10 Major aminoglycoside-modifying enzymes that act on kanamycin C. This aminoglycoside is susceptible to the largest number of enzymes. The *N*-acetyltransferases (AACs) affect amino functions and the *o*-nucleotidyltransferases affect hydroxyl functions. Each group of enzymes inactivates specific 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 aminoglycosides used clinically on which these enzymes act are 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* although clinical resistance was not conferred. Data from Shaw *et al.*[18]

Another approach has been to reduce aminoglycoside toxicity based on the underlying cellular and molecular mechanisms uncovered over the last 20 years.[21]

Tetracyclines and alkylaminocyclines

Chemical structure

The early tetracyclines were derived from *Streptomyces* spp. (tetracycline, oxytetracycline), in contrast to more recent semisynthetic compounds (doxycycline, minocycline). All are characterized by four hydrophobic fused rings, which are diversely substituted, but principally by oxygenated hydrophilic groups. Alkylaminocyclines possess an additional substituent with a bulky hydrophobic moiety and an ionizable amino function (Fig. 130.11). Tigecycline is often referred to as a glycylcycline based on the presence of a glycyl moiety as a spacer between the main part of the molecule and the ter-butyl-amino group. Because other derivatives with similar properties have different spacers but a similar hydrophobic amino group, a better name for the class is alkylaminocyclines.

Bacterial targeting

Tetracyclines penetrate the outer membrane of Gram-negative organisms through porins. Intracellular accumulation inside the bacteria depends on the pH gradient between the cytosol and the external medium, which may occur by diffusion or a transmembrane proton-
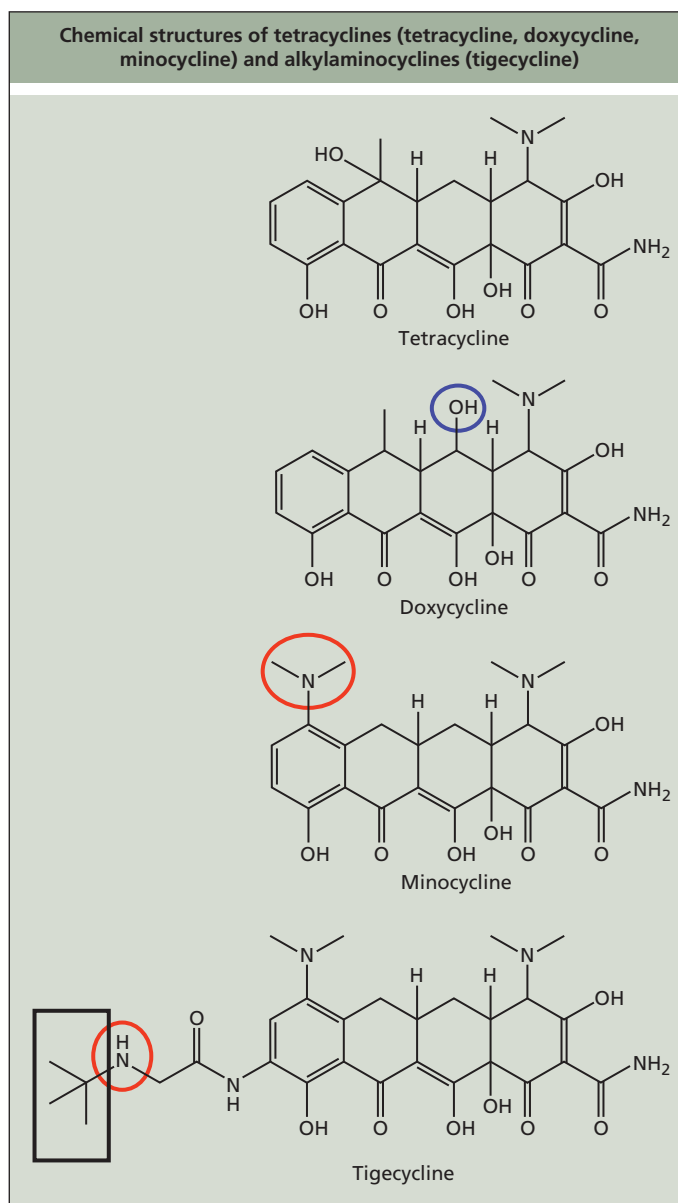
Fig. 130.11 Chemical structures of tetracyclines (tetracycline, doxycycline, minocycline) and alkylaminocyclines (tigecycline). The figure highlights the displacement of a hydroxyl group between tetracycline and doxycycline, and the additional ionizable aminofunction in minocycline which confer a more prolonged half-life and improved activity (minocycline). Tigecycline is the first of a new group of derivatives with a bulky hydrophobic moiety (square; *ter*-butyl in the case of tigecycline) and an additional amino group (circled) that make the molecule less susceptible to efflux by tetracycline–specific transporters as well as to ribosomal protection, the two main mechanisms of bacterial resistance towards all other tetracyclines.

driven carrier. The main argument in favoring the latter is that it could explain the selective action of tetracyclines (Fig. 130.12).

Mode of action

Tetracyclines interfere with the initiation step of protein synthesis (Fig. 130.12). More precisely, they inhibit the binding of aminoacyl tRNA to the A-site of the ribosome.[22] The 7S protein and the 16S RNA show the greatest affinity for tetracyclines and are therefore the main targets. This binding inhibits the fixation of a new aminoacyl tRNA on the ribosome. In addition, tetracyclines bind, or at least protrude, in the P-site by

Accumulation, antibacterial activity and efflux of tetracyclines
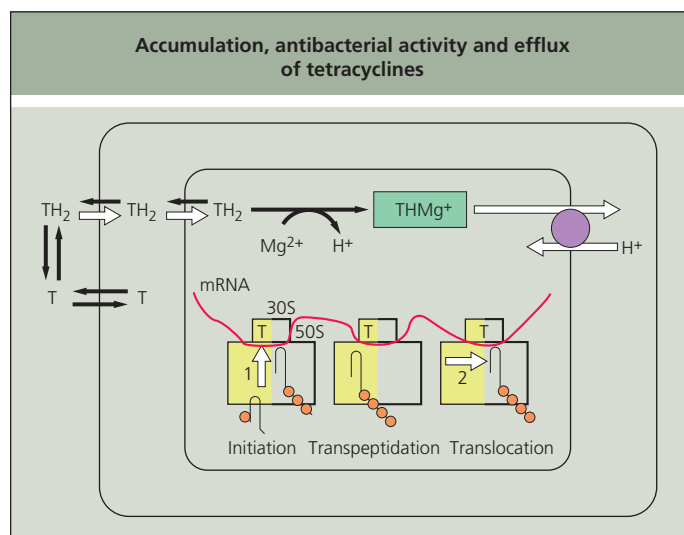
Fig. 130.12 Accumulation, antibacterial activity and efflux of tetracyclines. Tetracyclines freely diffuse through the extracellular membrane of Gram-negative organisms. Penetration inside bacteria is an energy-dependent process, depending on the pH and Mg^{2+} gradient between the extracellular medium of Gram-positive organisms or the periplasmic medium of Gram-negative organisms 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 (purple circle). The antibacterial action of the tetracyclines (T) 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 (yellow part of the ribosome). In the post-translocational state, tetracyclines protrude in the P-site (white part of the ribosome) and inhibit the binding of the peptidyl-tRNA (arrow 2).

alteration in ribosome conformation in the post-translocational state, and may modify the ribosome conformation at the level of the head of the 30S subunit and the interface side of the 50S subunit.

Resistance

Resistance to tetracyclines is widespread. Some 29 genes on mobile elements have been identified in the so-called 'tetracycline' (tet) family and three in the 'oxytetracycline resistance' gene family (otr).[22] Among the tet and otr genes, two main mechanisms have been described, namely efflux (which is an important and widespread mechanism of bacterial resistance, and was actually discovered with tetracyclines) and ribosomal protection. Resistance by enzymatic inactivation has been described but remains uncommon. Low levels of resistance can also result from mutations or a decrease in porin content of the outer membrane, with consequent decreased tetracycline uptake in Gram-negative bacteria.

As tigecycline increases the number of bonds to the target 16S RNA, the drug is unaffected by the ribosome protection mechanism.[23] Together with the vicinal hydrophobic moiety, it makes the molecule less susceptible to efflux, and is therefore active against many Gram-negative organisms with the exception of *P. aeruginosa* and Proteae because of a resistance nodulation cell division (RND)-type efflux pump (MexXY-OprM) constitutively expressed by these bacteria.[24]

Pharmacodynamics

Tetracyclines are essentially bacteriostatic and need to be administered at intervals determined by the drug half-life in order to maintain their serum level above the MIC of the infecting organism for as long as possible. Because of their prolonged half-life, however, AUC_{24h}/

MIC emerges as the main parameter driving the activity of tetracyclines (including tigecycline) *in vivo*. In contrast to conventional tetracyclines, tigecycline has no useful oral bioavailability and must be given by the intravenous route.

Future developments

Mutants resistant to tigecycline (mostly related to efflux) have already been reported in *Acinetobacter* spp. The development of efflux pump inhibitors would also be useful not only in this context but also to extend the activity of tigecycline and similar compounds against *P. aeruginosa*. Orally bioavailable alkylaminocyclines are also in development.

Fusidic acid

Chemical structure

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

Mode of action and resistance

Fusidic acid prevents the dissociation of the complex formed between guanosine diphosphate, elongation factor 2 and the ribosome, thereby inhibiting the translocation step of the peptidyl tRNA from the P-site to the A-site of the ribosome.[25] Because of a lack of cross-resistance with other antistaphylococcal agents, fusidic acid is enjoying a revival in several countries in the treatment of multiresistant *S. aureus*, but always in combination because of the high rate of emergence of resistance when used as monotherapy (mainly based on data from drug usage as a topical application; this could be much less if the drug is given orally).[26] The latter seems to result from the acquisition of the *fusB* gene (located on a transposon-like element of which homologues exist in many clinically important and environmental Gram-positive bacterial species), which protects ribosomal protein synthesis inhibition from fusidic acid in a dose-dependent fashion.[27]

Pharmacodynamics

Fusidic acid is bacteriostatic but may be bactericidal at high concentrations. Although fusidic acid has been used in many countries for years, it has never been approved in the US so far. In view of the mounting epidemic of community-acquired MRSA, a clinical development of fusidic acid in the US is presently under way.

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 have been called pseudomonic acids B, C and D.

Mode of action and resistance

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, leading to arrest of protein synthesis. Resistance to mupirocin develops through the production of a modified target enzyme.[28]

Because of its unique mechanism of action, there is no cross-resistance between mupirocin and other antimicrobial agents.[29]

Pharmacodynamics

Mupirocin is bacteriostatic at low concentration but becomes bactericidal at concentrations achieved locally by topical administration. The *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.

Future developments

The peculiar mode of action of mupirocin has triggered genomic-based research to identify similar targets at the level of the other amino acids, which could lead to new compounds.

Retapamulin

Retapamulin is a semisynthetic derivative of pleuromutilin, a naturally occurring tricyclic antibiotic, diterpene, discovered in the early 1950s, and out of which only veterinary antibiotics had been developed until now. Retapamulin inhibits bacterial protein synthesis by binding to domain V of 23S rRNA, thereby blocking peptide formation directly by interfering with substrate binding. Resistance occurs through mutations in the genes encoding 23S rRNA, but is not crossed with other antibiotics as the binding site is unique compared with those of other antibiotics acting on ribosomes.[30] This makes retapamulin appealing for the treatment of a variety of susceptible Gram-positive pathogens. Retapamulin has been developed as a topical antibiotic for the management of impetigo and uncomplicated secondarily infected traumatic skin lesions.

Macrolides

Chemical structure

The main active macrolides are 14-, 15- or 16-membered lactone rings, substituted by two sugars, one of which bears an aminated function. In 15-membered macrolides (azithromycin), an additional aminated function is inserted in the lactone ring, conferring to this subclass of molecule the name of 'azalides'. Ketolides are 14-membered macrolides in which the cladinose is replaced by a keto function and which possess in their macrocycle a carbamate linked to an alkyl-aryl extension, represented by telithromycin as the only registered antibiotic (Fig. 130.13).[31]

Erythromycin, the first clinically developed macrolide, is a natural product. Most of the more recent molecules are semisynthetic derivatives designed to be stable in acidic milieu, and are therefore characterized by an improved oral bioavailability. Both 16-membered macrolides and ketolides are intrinsically acid stable.

Bacterial targeting

Macrolides specifically bind to the 50S subunit of the ribosomes (more precisely, to the 23S rRNA), which does not exist in eukaryotic cells.

Mode of action

Macrolides reversibly bind to the peptidyl transferase center, located at the 50S surface, causing multiple alterations of the 50S subunit functions. While macrolides only bind to domain V of the 23S rRNA, ketolides also bind to domain II of 23S rRNA as a result of their carbamate extension, and thus are double anchored to their target (Fig. 130.14).[32] Macrolides are classically thought to block the peptide bond formation or the peptidyl tRNA translocation from the A-site to the P-site. However, additional consequences of their binding to ribosomes have been reported. It has been proposed 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.[33] It has also been suggested that erythromycin prevents the assembly of the 50S subunit, a property not generalizable to other macrolides.

Resistance

Clinically meaningful resistance occurs primarily by modification of the bacterial target and therefore affects all macrolides; it can also occur by efflux.[34] Target modification also affects lincosamides and streptogramins because of the common binding site for these three classes of antibiotic and also explains why macrolides, streptogramins, lincosamides and chloramphenicol have antagonist pharmacologic activity. Resistance to macrolides may be inducible or constitutive; however, if inducible it will not affect streptogramins and lincosamides since these are not inducers.[34] Ketolides (due to their lack of cladinose) and 16-membered macrolides are not inducers and therefore show activity on a subset of resistant strains. Moreover, the double ribosomal anchoring of ketolides confers a higher affinity not only for wild-type ribosomes, but also for ribosomes of strains resistant by methylation of domain V, with consequent improved activity against resistant strains.[31]

Although efflux mechanisms are being reported, 16-membered macrolides are again spared this effect. The frequency of strains susceptible to 16-membered macrolides and resistant to 14- and 15-membered macrolides remains low, however.

Pharmacodynamics

Macrolides are essentially bacteriostatic antibiotics, except at high concentrations. Their concentration at the infected site therefore needs to be durably maintained above the MIC of the pathogen. Because of their prolonged half-life, clarithromycin, azithromycin and telithromycin have been shown to primarily reflect the AUC_{24h}/MIC parameter *in vivo*.[31]

Future developments

Efforts to develop new ketolides continue, with the aim of selecting compounds with improved intrinsic activity (especially against multi-resistant pneumococci and community-acquired MRSA) and a better safety profile, as telithromycin has been severely restricted in its indications because of liver toxicity.[31]

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.

Resistance

The main mechanism of resistance to lincosamides is similar to that of macrolides and streptogramins and consists of an alteration of the 50S subunit. Rare cases of enzymatic inactivation (adenylation reaction) of the antibiotic have also been described for clindamycin. Resistance dissociation is, however, observed for those strains for which macrolide resistance is inducible, as clindamycin is not an inducer.[35] Likewise, efflux-mediated resistance to macrolides does not affect lincosamides, also leading to resistance dissociation.[34]

Pharmacodynamics

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

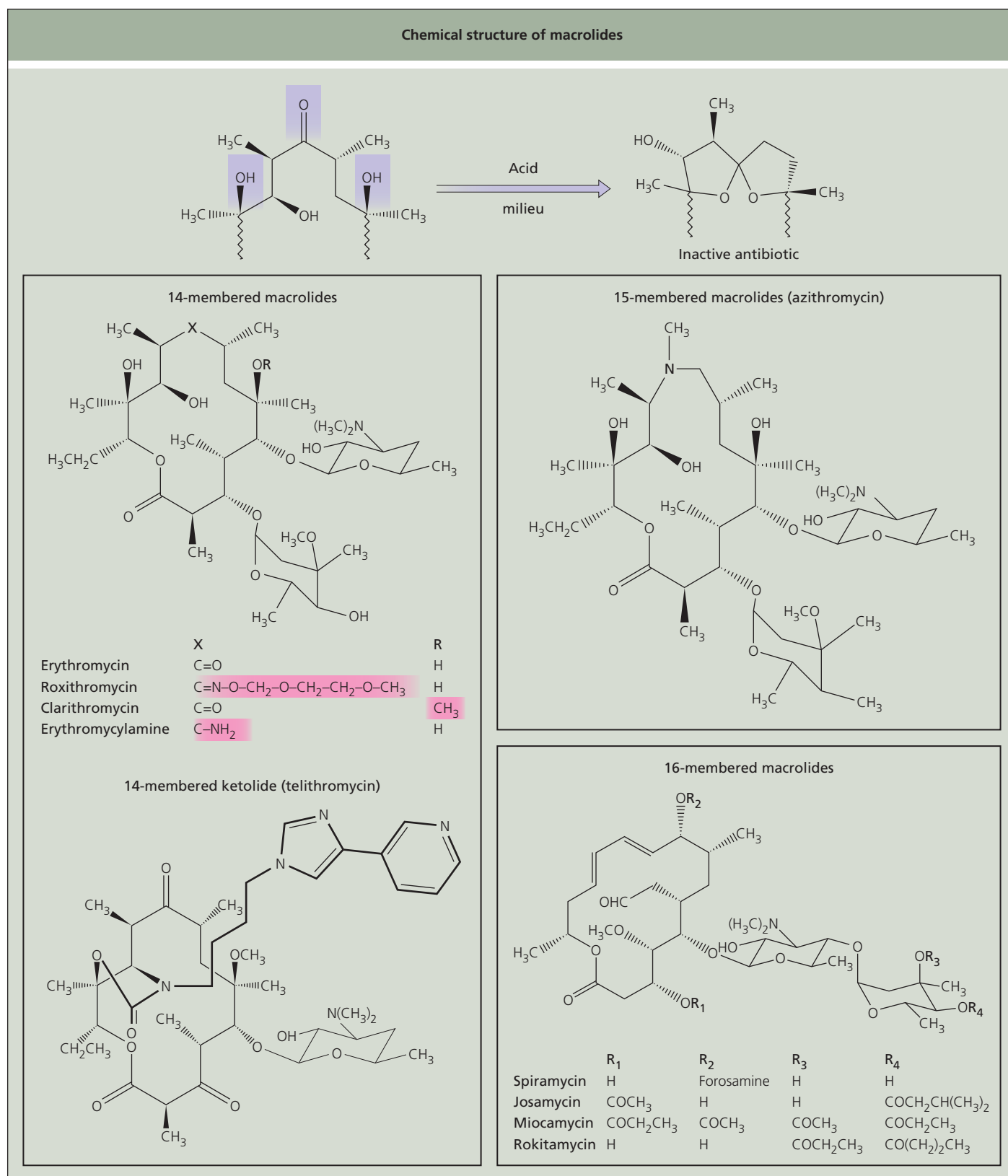Fig. 130.13 Chemical structure of the macrolides. The upper panel shows the degradation of erythromycin in the gastric milieu (substituents responsible for the instability of the miolecule are shown in purple). 16-Membered macrolides and ketolides are intrinsically stable. The structural modifications conferring stability in acidic milieu to 14- and 15-membered macrolides are highlighted in pink in the middle panel.
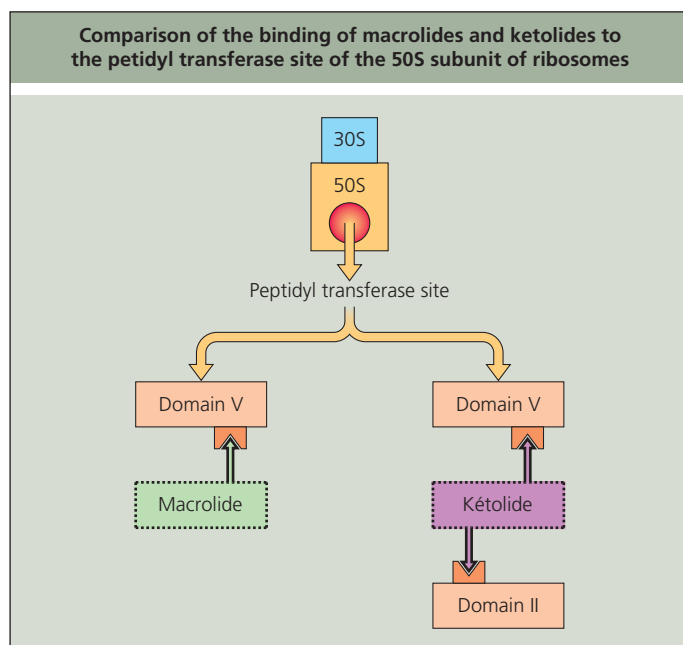
Comparison of the binding of macrolides and ketolides to the petidyl transferase site of the 50S subunit of ribosomes

Fig. 130.14 Comparison of the binding of macrolides and ketolides to the peptidyl transferase site of the 50S subunit of ribosomes. Macrolides are characterized by a single anchoring point and ketolides by a double anchoring point, which increases the affinity of ketolides for wild type and methylated ribosomes.

Streptogramins

Chemical structure

Streptogramins are antibiotics that comprise a pair of synergistic constituents, namely a depsipeptide (group I) and a lactonic macrocycle (group II). Quinupristin–dalfopristin is the only combination used in the clinic so far.[36]

Mode of action

Streptogramins bind to the 50S subunit of the bacterial ribosome. They interfere with protein synthesis by a double mechanism, involving inhibition of the incorporation of aminoacyl tRNA in the ribosome and the translation of mRNA. The synergistic effects of the two components could be due to a modification of the conformation of the ribosome caused by binding of the group I component, which exposes a site of fixation for the group II component.[33]

Resistance

Resistance is by mutation of the ribosomal target and results in cross-resistance with macrolides and lincosamides. Because of the presence of two components, however, synergistins remain active against many macrolide and lincosaminide-resistant isolates. Resistance to streptogramins alone is rare and occurs by enzymatic inactivation (hydrolase and acetylase).

Pharmacodynamics

Streptogramin constituents are highly synergistic and exhibit dose-dependent bactericidal activity in combination. In addition, they also increase the antibiotic activity of aminoglycosides and rifamycins. Streptogramins also exhibit a prolonged postantibiotic effect with delayed regrowth when the antibiotic concentration falls under its MIC. This may result from persistent binding of the drug to its target.

Future developments

Quinupristin/dalfopristin currently has limited use only. There is, however, a potential interest in novel streptogramins for the treatment of infections caused by bacteria resistant to other antibiotics, mainly multiresistant *S. aureus* and vancomycin-resistant enterococci. At least one compound for oral administration (NX-103) is under development.

Chloramphenicol and thiamphenicol

Chemical structure

These antibiotics are constructed on a dichloroacetamide bearing a diversely substituted phenyl group.

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 is due to competitive inhibition for the binding of aminoacyl tRNA to the peptidyltransferase domain of the 50S subunit. This induces conformational change in the ribosome, which slows or even inhibits the incorporation of the aminoacyl tRNA and in turn the transpeptidation reaction.[37]

Resistance

Resistance to chloramphenicol is mainly due to the production of a specific inactivating acetyltransferase.[38] The encoding gene is often located on plasmids that also confer resistance to other antibiotic classes. Another mechanism of resistance results in reduced drug entry into the bacterium.

Pharmacodynamics

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

Oxazolidinones

Chemical structure

Like fluoroquinolones, oxazolidinones are synthetic molecules. The first derivatives were described in the 1970s, although linezolid, the first clinically available molecule, only became available in the 1990s (Fig. 130.15). The 5-(S)-configuration of the oxazolidinone ring is essential for activity, which is further improved by its substitution by an *N*-fluorinated aryl group and a C5 acylaminomethyl group.[39]

Mode of action

Oxazolidinones inhibit protein synthesis at an earlier step than other antibiotics acting on the ribosome. Their binding site is located in the vicinity of the peptidyl transferase with the A-site of the bacterial ribosome where they seem to interfere with the placement of the aminoacyl tRNA.[40] This interaction prevents the formation of the initiation ternary complex which associates tRNA^{met}, mRNA and the 50S subunit of the ribosome, and therefore the binding to the ribosome as well as the synthesis of peptide bonds, and the translocation of tRNA^{met} into the P-site. They can compete for binding to the 50S subunit with other antibiotics (e.g. lincosamides, chloramphenicol) without being antagonistic.

Linezolid, the only available oxazolidinone, is mainly active against Gram-positive cocci, with minimal activity against most Gram-negative bacteria as a result of drug efflux in these organisms.[41]
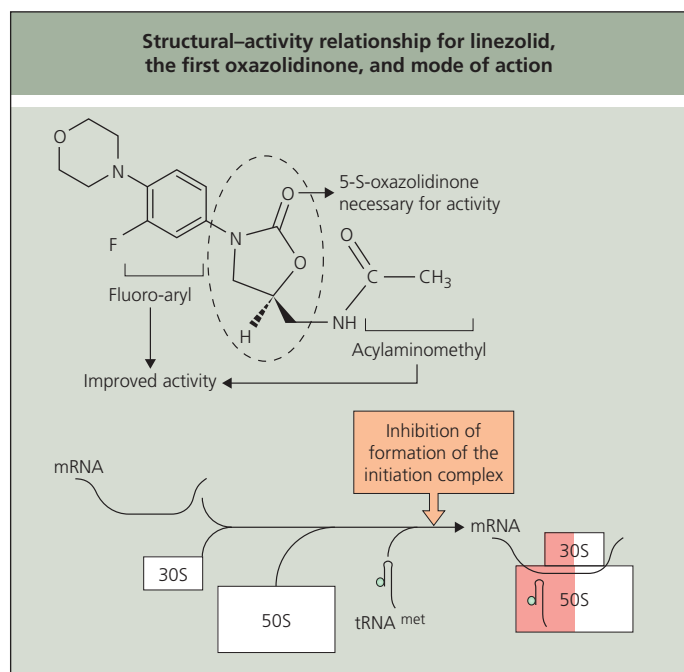
Fig. 130.15 Structure–activity relationship for linezolid, the first oxazolidinone, and mode of action. The drug prevents the formation of the ternary complex between mRNA, ribosome subunits and tRNA^{met} necessary for protein synthesis.

Oxazolidinones also interact with mitochondrial ribosomes to inhibit protein synthesis, which is probably the basis for the undesirable myelosuppressive effects of linezolid.[42]

Resistance

Because of the unique mode of action of oxazolidinones, there is no cross-resistance with other antibiotics acting on protein synthesis. Resistance develops following point mutations of the 23S rRNA[43] or deletions in the gene encoding riboprotein L4.[44]

Pharmacodynamics

Oxazolidinones are bacteriostatic against enterococci and staphylococci with a short postantibiotic effect. For streptococci, linezolid is bactericidal for the majority of strains. Because of its prolonged half-life, linezolid activity is primarily dependent on the AUC_{24h}/MIC parameter *in vivo*.[11]

Future developments

New oxazolidinones being developed have a broadened spectrum, increasing intrinsic activity and, potentially, reduced side-effects, and no new compounds (torezolid and radezolid) are currently in clinical development.

DRUGS THAT AFFECT NUCLEIC ACIDS (DNA/RNA)

Fluoroquinolones

Chemical structure

Fluoroquinolones are totally synthetic products originally derived from nalidixic acid. All current compounds have a dual ring structure,

with a nitrogen at position 1, a free carboxylate at position 3 and a carbonyl at position 4. A fluor substituent at position 6 usually greatly enhances activity, whereas the substituents at positions 7, 8 and N1 modulate the spectrum, the pharmacokinetics and the side-effects of the drugs (Fig. 130.16).[45] In this respect, more recent molecules (moxifloxacin, gemifloxacin, gatifloxacin) have been designed to better cover Gram-positive organisms, while retaining activity against Gram-negative organisms and, in the case of moxifloxacin, against anaerobes. They all present a small hydrophobic substituent at N1 and a diaminated small-sized ring substituent at position 7.

Bacterial targeting

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

Mode of action

Fluoroquinolones inhibit the activity of topoisomerases. These enzymes are responsible for the supercoiling of DNA (DNA gyrase) and the relaxation of supercoiled DNA (topoisomerase IV). Both enzymes have a similar mode of action, implying:

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

Gyrase and topoisomerase IV are tetramers composed of two types of subunit, namely two GyrA or ParC catalyzing the DNA cutting and resealing, and two GyrB or ParE responsible for the transduction and binding of ATP. The main target of fluoroquinolones is DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive bacteria.[46]

Fluoroquinolones form a ternary complex with DNA and the enzyme (Fig. 130.17).[47] This binding site for fluoroquinolones is formed during the gate-opening step of the double-stranded DNA. Co-operatively, four fluoroquinolone molecules are fixed to single-stranded DNA. Their stacking is favored by the presence of the co-planar 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 the carbonyl and carboxylate groups. Interaction with the enzyme is mediated by fluoride at position 6 and the substituents at position 7. The binding of the fluoroquinolones stabilizes the cleavable complex (formed by the cut DNA and the enzyme) and leads to the dissociation of the enzyme subunits. The latter action is, however, observed only for potent molecules or at higher concentrations.

Fluoroquinolones are highly bactericidal, which is not explained by the above mechanisms alone and requires RNA and protein synthesis to be observed, suggesting the formation of abnormal proteins as a consequence of DNA cleavage. Quinolones also induce an SOS (DNA repair) response, which involves three proteins (RecA, LexA and RecBCD). Induced RecA cleaves the repressor 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, leading to mutagenesis as well as increased cell survival in the presence of quinolones. This system therefore protects against the antibacterial activity of fluoroquinolones and could be a basis for emergence of resistance.[48]

Resistance

Resistance was long considered to be only chromosomally mediated from mutation of the topoisomerases (reducing drug-binding ability), porin impermeabilization (for Gram-negative bacteria) or
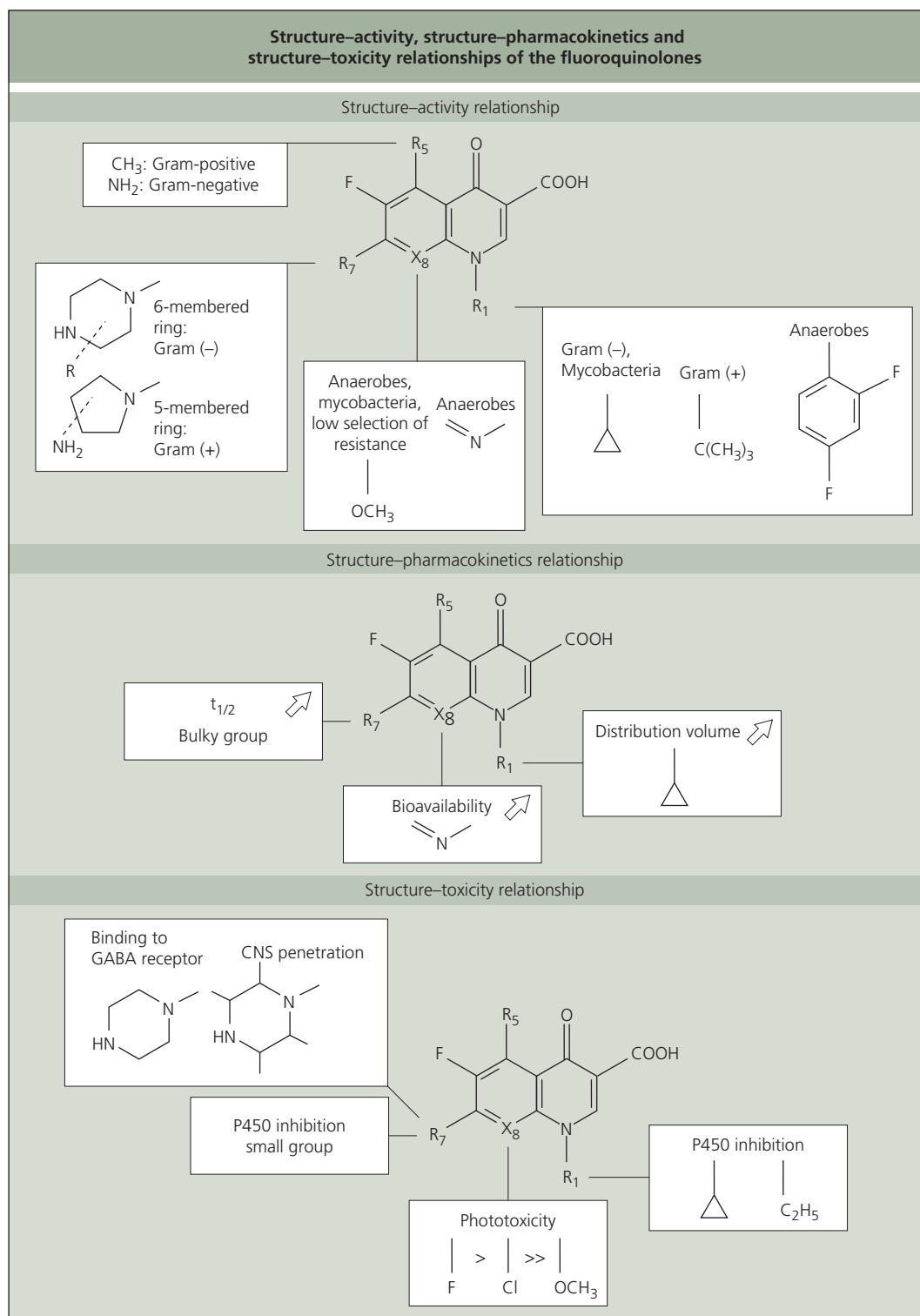
Fig. 130.16 Structure–activity, structure–pharmacokinetics and structure–toxicity relationships of the fluoroquinolones. These considerations form the basis of the rational development of the new molecules of this class, which have a very extended spectrum (including Gram-positive bacteria and anaerobes), a long half-life and minimal phototoxicity and metabolic interactions.

efflux.[45] A methoxy substituent at position 8 (see Fig. 130.16) lowers the ratio of the MIC in gyrase mutant strains to the corresponding MIC in the wild type, thereby reducing the risk of selecting resistant mutants during therapy. Plasmid-mediated resistance, however, is now increasingly observed,[49] related to the production of:

- Qnr proteins capable of protecting DNA gyrase from quinolones (which have homologues in water-dwelling bacteria where they probably serve as chaperones); and
- AAC(6′)-Ib-cr, a variant aminoglycoside acetyltransferase capable of acetylating ciprofloxacin.

Pharmacodynamics

The activity of fluoroquinolones is largely both concentration dependent (which drives the bactericidal effect) and proportional to the amount of drug administered (which drives the global efficacy *in vivo*), making these drugs C_{max}/MIC and AUC_{24h}/MIC dependent for their activity. For older fluoroquinolones that have a short half-life (e.g. ciprofloxacin), this imposes the use of repeated doses per day (combining all doses in a single administration would favor bactericidal effects but would risk toxic reactions). For newer fluoroquinolones with more prolonged

Fig. 130.17 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 shows the parts of the antibiotic molecules interacting with DNA, with the enzyme, or favoring the stacking of the fluoroquinolone molecules. Adapted from Shen *et al.*[47]

Ternary complex formed between DNA, DNA–gyrase or –topoisomerase IV and stacked fluoroquinolones
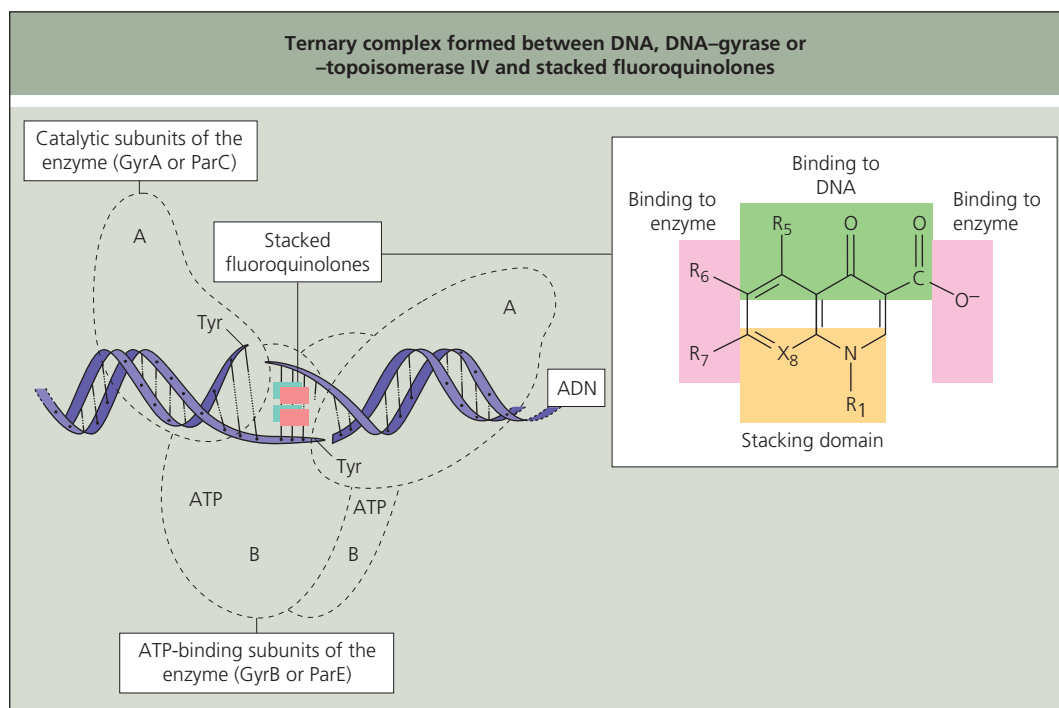
half-lives (levofloxacin, moxifloxacin, gemifloxacin), once-daily administration is possible, with the aim of obtaining both C_{max}/MIC (>8) and AUC_{24h}/MIC (from 30 to 125 or more) ratios, offering optimal efficacy and minimizing selection of resistant subpopulations.[45,50] The European breakpoints (usually ≤2 mg/l) have taken this into account.

Future developments

New molecules with extended spectra, still greater intrinsic activity and resistance to efflux transporters are being sought, although a major difficulty is to avoid increases in toxicity. Molecules with enhanced activity at acid pH (for urinary tract, intracellular or stomach infections) or much lower MICs than registered fluroquinolones are currently being developed.

Nitroimidazoles and nitrofurans

Chemical structure

The nitroheterocyclic drugs include nitrofuran and nitroimidazole compounds (Fig. 130.18).

Mode and spectrum of action

The activity of the nitroheterocyclic drugs requires activation of the nitro group attached to the imidazole or furan ring, which must undergo single- or two-electron enzymatic reduction in the bacteria.[51] Although the nitro radicals generated by reduction of the parent drugs are similar for the nitroimidazoles and the nitrofurans, these drugs differ by their reduction potential, and therefore their spectrum of activity. Thus nitroimidazoles must be fully reduced to generate the highly reactive species (hydroxylamines) that cause damage, whereas singly reduced nitrofurans may directly inhibit the activity of enzymes involved in the degradation of glucose and pyruvate and covalently bind to proteins and DNA by an alkylation reaction. Nitroimidazoles will, therefore, express activity only towards anaerobic and microaerophilic bacteria, and to other parasitic organisms such as *Trichomonas vaginalis*, capable of generating a sufficiently low redox potential thanks to the

presence of an H_2-generating organelle (hydrogenosome), whereas nitrofuranes are equally active against anaerobic and aerobic bacteria.

Resistance

Resistance to nitroimidazoles in true anaerobic bacteria is rare, but has been described in *Bacteroides fragilis* (combination of decreased antibiotic uptake, reduced nitroreductase and pyruvate:ferredoxin oxidoreductase activity and increased lactate dehydrogenase activity).[52] It has become significant in *Helicobacter pylori* (null mutations in *rdxA* encoding an oxygen-insensitive nitroreductase that normally prevents reoxidization of metronidazole in the microaerophilic environment of this bacterium).[53]

Pharmacodynamics

Nitroimidazoles show concentration-dependent killing, which is consistent with the current clinical pattern of dispensing a large dose in a single administration (although more frequent administration of lower dose is also recommended).

Future developments

The variety of substitutions that can be attached to the ring structures may allow new drug development. This has not been explored so far for antibacterial therapy, although finding new derivatives for amebiasis and giardiasis is being actively pursued.

Ansamycins

Chemical structure

These 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. The first clinically developed and major antibiotic in this class is rifampin (rifampicin). Successful successors have been rifapentin, rifamixin and rifabutin.
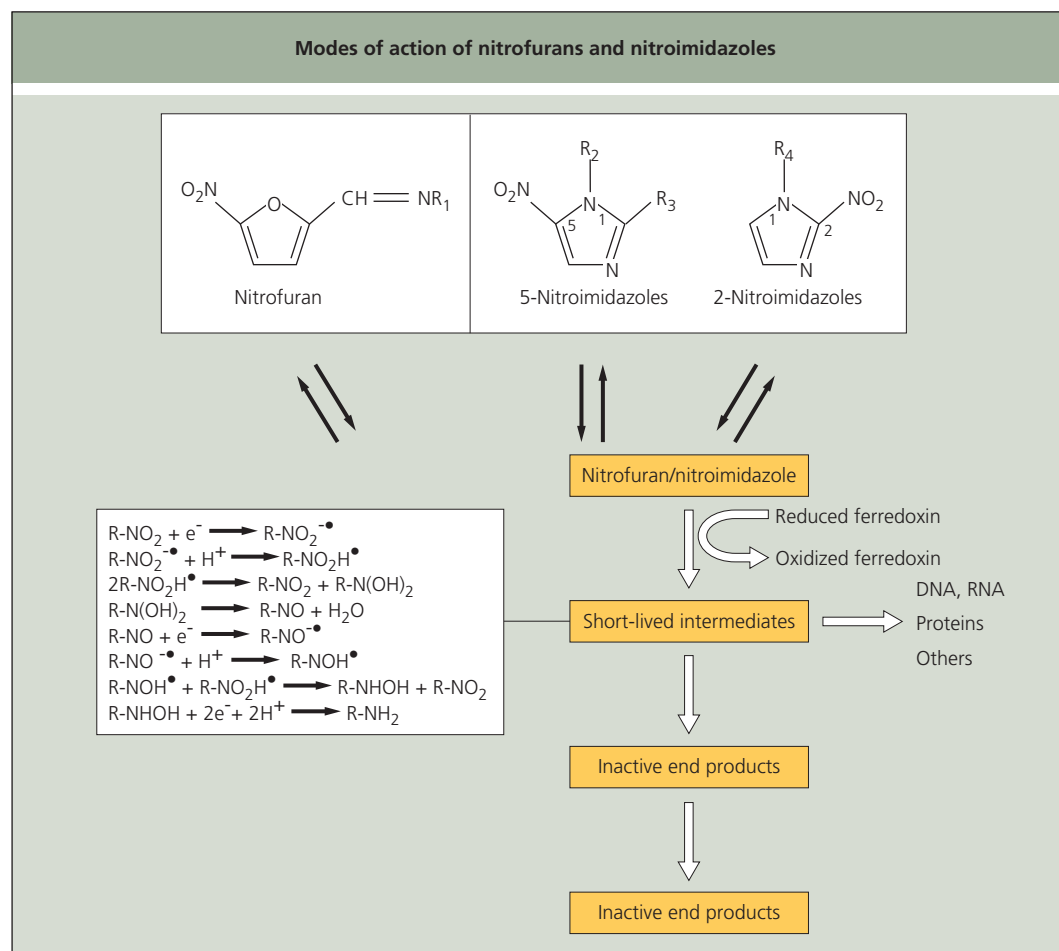
Fig. 130.18 Structures and modes of action of nitrofurans and nitroimidazoles. The molecules must be reduced to form highly reactive products that interact with intracellular targets.

The reactions shown in the figure:

$$R\text{-}NO_2 + e^- \longrightarrow R\text{-}NO_2^{-\bullet}$$
$$R\text{-}NO_2^{-\bullet} + H^+ \longrightarrow R\text{-}NO_2H^\bullet$$
$$2R\text{-}NO_2H^\bullet \longrightarrow R\text{-}NO_2 + R\text{-}N(OH)_2$$
$$R\text{-}N(OH)_2 \longrightarrow R\text{-}NO + H_2O$$
$$R\text{-}NO + e^- \longrightarrow R\text{-}NO^{-\bullet}$$
$$R\text{-}NO^{-\bullet} + H^+ \longrightarrow R\text{-}NOH^\bullet$$
$$R\text{-}NOH^\bullet + R\text{-}NO_2H^\bullet \longrightarrow R\text{-}NHOH + R\text{-}NO_2$$
$$R\text{-}NHOH + 2e^- + 2H^+ \longrightarrow R\text{-}NH_2$$

Mode of action

Ansamycins inhibit the initiation of the transcription of DNA in mRNA and therefore subsequent protein synthesis. The RNA polymerase is made up of 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 initiates transcription and then leaves the polymerase nucleus.

The core polymerase ($\alpha_2\beta\beta'$) therefore retains the capacity 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. All the mutations affecting drug binding belong to three clusters of amino acids in the central domain of the β subunit. 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. 130.19).

Pharmacodynamics

Rifamycins are bactericidal. This effect could be due to either the high stability of the complex formed between rifampin and the enzyme or the formation of superoxide ions of the quinone ring of the antibiotic molecule. Because their action is to hinder bacterial multiplication,
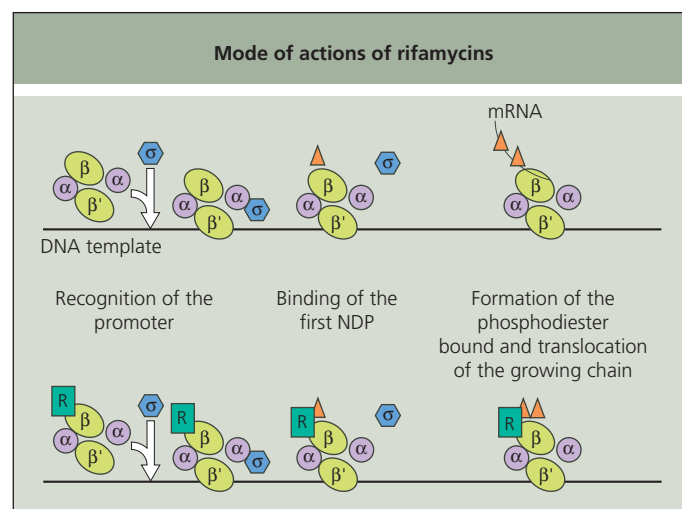
Fig. 130.19 Mode of action of rifamycins. Synthesis of mRNA by RNA polymerase is shown in the upper panel and inhibition by rifamycins (R in the green squares) is shown in the lower panel. The RNA polymerase core is made up of four subunits, of which the β' subunit binds to the DNA template and the β subunit binds the ribonucleotide diphosphate (NDP; triangle). The σ 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.

they are, at least *in vitro*, antagonists to antibiotics requiring active bacterial growth to exert their activity (β-lactams) or to other antibiotics acting 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 vs extracellular for β-lactams and aminoglycosides).

Rifamycins show a long postantibiotic effect because of the irreversible character of their binding. The excellent penetration of rifamycins in eukaryotic cells has often been a major argument for supporting their use against intracellular organisms, including mycobacteria (exposure to critical concentrations of rifampin may be sufficient to kill intermittently metabolizing mycobacterial populations).[54]

Future developments

Benzoxazinorifamycins constitute a new group of semisynthetic molecules with development oriented towards *Chlamydia* infections and other intracellular organisms.[55] Of note, diarylquinolines, inhibitors of mycobacterial ATP synthase,[56] have now emerged as novel tuberculostatic agents acting even on dormant organisms, with TMC207 (R207910) having reached a clinical stage.

ANTIMETABOLITES

Sulfonamides and diaminopyrimidines

Prontosil (sulfamidochrysoidine) – a synthetic compound with antibacterial activity found by Domagk in 1932 – was a prodrug which led to the development of the sulfonamides. With diaminopyrimidines, they inhibit the folate pathway in bacteria. For many years, diaminopyrimidines were used in combination with sulfonamides except for specific indications (parasitic diseases) or for the treatment of uncomplicated cystitis. A recent revival of diaminopyrimidines has been the discovery and development of iclaprim (not yet approved).

Chemical structure

Sulfonamides are derived from *p*-aminobenzenesulfonamide which is a structural analogue of *p*-aminobenzoic acid, a factor required by bacteria for folic acid synthesis (Fig. 130.20). A free amino group at position 4 and a sulfonamide group at position 1 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 (Fig. 130.20) and pyrimethamine are pyrimidines substituted at position 5 by an aromatic group. Pyrimethamine has an additional substituent at position 6. Iclaprim (Fig. 130.20) is the result of pharmacochemical research aimed at selecting compounds not only more active than trimethoprim but also expanding activity against trimethoprim-resistant strains. It is a racemic mixture of two enantiomers, both of which show equipotent activity.

Mode of action

Sulfonamides inhibit tetrahydrofolic acid synthesis, acting at the level of dihydropteroate synthetase as analogues of *p*-aminobenzoic acid, and as alternative substrates to become incorporated into a product with pteridine.[57,58]

Diaminopyrimidines are specific competitive inhibitors of bacterial dihydrofolate reductase.[57,58] Selectivity of action is achieved as trimethoprim in establishing more binding interactions with the bacterial than with the corresponding eukaryotic enzymes. In addition, the NADPH co-factor may stabilize the enzyme–trimethoprim complex in bacteria. Iclaprim shows additional increased binding and the ability to inhibit the trimethoprim-resistant enzyme, based on the lipophilic interactions of the cyclopropyl ring with two hydrophobic amino acids (Leu54 and Ile50) of the target enzyme.[59]

Structures of *p*-aminobenzoic acid and sulfamethoxazole (top) and trimethoprim and iclaprim (bottom)

p-aminobenzoic acid

Sulfamethoxazole

Dihydrofolic acid

Trimethoprim
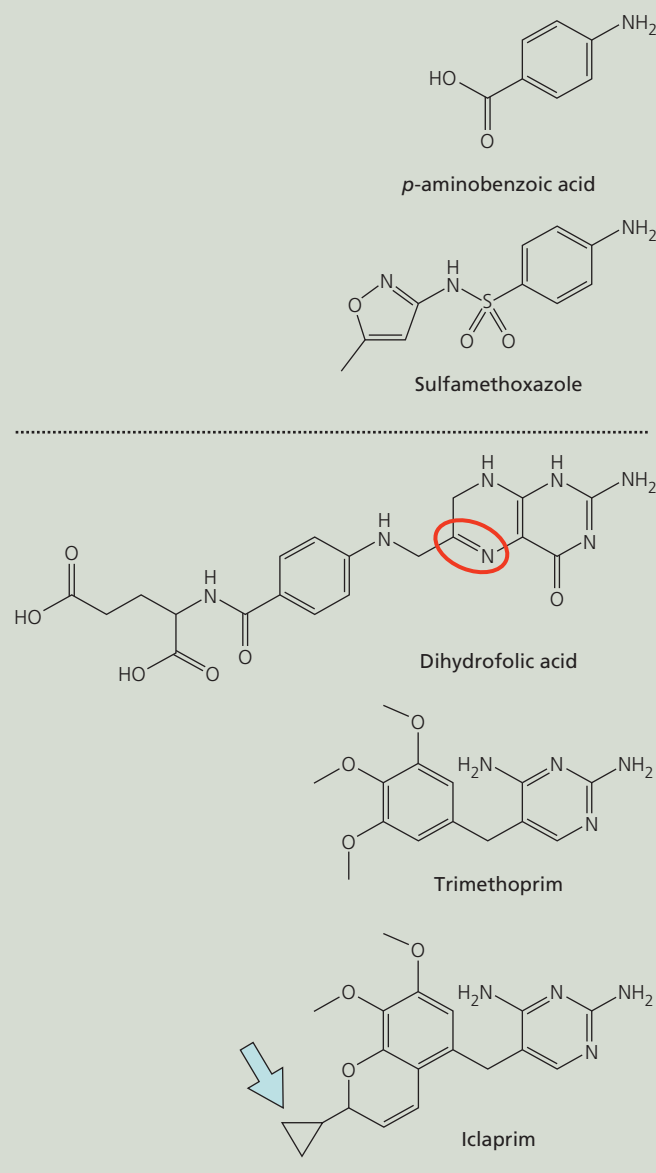
Iclaprim

Fig. 130.20 Structures of *p*-aminobenzoic acid and sulfamethoxazole, and trimethoprim and iclaprim. The figure highlights the similarity between *p*-aminobenzoic acid (a sulfonamide) and dihydrofolic acid and trimethoprim, which are the basis for the activity of these drugs (in dihydrofolic acid, the red circle outlines the positions that are reduced for conversion into tetrahydrofolic acid); this part of the figure also shows the differences between trimethoprim and iclaprim, for which the bulky cyclopropyl moiety (block arrow) strengthens the interactions with the target enzyme and helps to overcome resistance to trimethoprim.

Resistance

For sulfonamides, resistance mainly occurs from hyperproduction of *p*-aminobenzoic acid or a reduced affinity of dihydrofolate reductase for the antibiotic, causing resistance to the whole class. For diaminopyrimidines, resistance mostly occurs via enzyme mutations which prevent binding, but this can be reversed by suitable modification such as that made with iclaprim.[59]

Pharmacodynamics

Sulfonamides are bacteriostatic; however, in combination with diaminopyrimidines they are bactericidal. The pharmacodynamics of iclaprim are poorly defined, mainly because of low blood levels and interference by thymidine on its activity.

ANTIBIOTICS ACTING ON THE MEMBRANE

Cyclic polypeptides (polymyxins/colistins)

Chemical structure

These are a collection of cyclic, branched polypeptides (molecular masses about 1000 Da) containing both cationic and hydrophobic amino acids. Some of these are of the D configuration or are non-DNA coded, which confers resistance to mammalian peptide-degrading enzymes. Polymyxins are obtained from *Bacillus polymyxa* and colistins from *Aerobacillus colistinus*. Only polymyxin B and colistin (identical to polymyxin E) are used in clinical practice. Commercial colistin contains at least two components (E1 and E2, also called colistin A and colistin B) differing by the length of the fatty acid chain.

Mode of action

Because of their amphipathic character, polymyxins and colistins act as detergents and alter the permeability of the cytoplasmic membrane.[60] They therefore act at all stages of bacterial development. However, they cannot diffuse easily through the thick peptidoglycan layer of Gram-positive bacteria. In contrast, they bind easily to the outer membrane of Gram-negative bacteria (interacting with the lipopolysaccharide (LPS) and triggering a 'self-promoted uptake' process) from where they reach the cytoplasmic membrane through polar as well as nonpolar channels. These properties explain their strong and fast bactericidal activity through disruption of the inner membrane and their essentially Gram-negative spectrum.

Resistance

Acquired resistance to polymyxins and colistins is chromosomal and results from decreased permeability of the outer membrane secondary to changes in its biochemical composition. Bacteria with decreased sensitivity are characterized by a decreased phospholipid/lipid ratio and a higher content of divalent cations (Ca^{2+}, Mg^{2+}). Protein H1 from *P. aeruginosa* (OprH) prevents binding of polymyxins and colistins to lipopolysaccharide, and its overproduction correlates with reduced sensitivity. However, this change is not sufficient per se and must be combined with other modifications of the membrane; two genes downstream to OprH (PhoP and PhoQ) co-regulate OprH and polymyxin B resistance. Resistance to polymyxins and colistins was previously uncommon but is now increasingly described in strains exhibiting multiple resistance to β-lactams and aminoglycosides. A puzzling observation is that the regrowth is easily observed *in vitro*, suggesting the occurrence of a so-called 'adaptive resistance', the mechanism of which is still uncertain.

Pharmacodynamics

Colistin A and polymyxin B show concentration-dependent activity but little or no postantibiotic effect, justifying the administration of repeated daily doses.[61] The pharmacodynamic parameters governing the activity of colistin are still undefined as both C_{max}/MIC and time above MIC are critical not only for efficacy but also to prevent regrowth.

Nonantibiotic pharmacologic and toxicologic 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 are potentially useful, such as inactivation of endotoxins and synergy with serum bactericidal activities. Others, however, are highly detrimental to the host and include activation of the alternate complement pathway, mastocyte degranulation with histamine release, decreased production of cytokines (but increased tumor necrosis factor release), increased membrane conductance in epithelia, and apoptosis.

Future developments

Because of the widespread emergence of resistance to other antimicrobials, colistin B has become more frequently used in chronic, difficult-to-treat infections (e.g. pulmonary infections in cystic fibrosis patients). Polymyxin B has also been investigated as an anti-endotoxin agent.[54] New, potentially less-toxic derivatives of these molecules and other membrane-destabilizing peptides have also been isolated or synthesized recently with some of them being under clinical development.[53]

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