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Review

Lincosamides: Chemical structure, biosynthesis, mechanism of action, resistance, and applications

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ABSTRACT

Lincomycin and its derivatives are antibiotics exhibiting biological activity against bacteria, especially Gram-positive ones, and also protozoans. Lincomycin and its semi-synthetic chlorinated derivative clindamycin are widely used in clinical practice. Both antibiotics are bacteriostatic, inhibiting protein synthesis in sensitive bacteria; however, at higher concentrations, they may be bactericidal. Clindamycin is usually much more active than lincomycin in the treatment of bacterial infections, in particular those caused by anaerobic species; it can also be used for the treatment of important protozoal diseases, *e.g.* malaria, most effectively in combination with other antibiotic or non-antibiotic antimicrobials (primaquine, fosfidomycin, benzoyl peroxide). Chemical structures of lincosamide antibiotics and the biosynthesis of lincomycin and its genetic control have been summarized and described. Resistance to lincomycin and clindamycin may be caused by methylation of 23S ribosomal RNA, modification of the antibiotics by specific enzymes or active efflux from the bacterial cell.

1. Introduction

The term lincomycin is based on Lincoln, Nebrasca, where the antibiotic was first isolated from *Streptomyces lincolnensis* in a soil sample. Lincosamides constitute a relatively small group of antibiotics with a chemical structure consisting of amino acid and sugar moieties. The naturally occurring members of the group are lincomycin and celesticetin, the latter exhibiting 5% of the biological activity of lincomycin *in vivo*. Many semisynthetic derivatives of lincomycin have been prepared. Of these, only the chlorinated derivative clindamycin is highly biologically active and is applied in clinical practice. Natural lincosamides are produced by several *Streptomyces* species, mainly by *S. lincolnensis*, *S. roseolus*and *S. caelestis* and by *Micromonospora halophytica* and act by inhibiting protein synthesis in sensitive microorganisms. . Lincosamides are widely and potently active against anaerobic bacteria and some protozoa. The major way of resistance to lincosamides is modification of the 23S rRNA in the 50S ribosomal subunit, similarly to the resistance against macrolides and streptogramin B (MLS_b resistance). Clindamycin is the main lincosamide antibiotic applied in clinical practice.

2. Chemical structure of lincosamides

Lincomycin consists of an unusual amino acid, *viz.* *trans*-*N*-methyl-4-*n*-L-proline (propylhygric acid) linked by a peptide bond with the sugar 6-amino-6,8-dideoxy-1-thio-D-*erythro*-α-D-galactopyranoside (methylthio-lincosamide) [1,2]. Natural and semisynthetic lincosamides are lincomycins A, B, C, D, S, K, celesticetins A, B, C, D, desalicetin, desalicetin D, and N-demethylcelesticetin; the most important semi-synthetic derivative with high biological activity is the chlorinated derivative clindamycin [3]. Like with other promising antibiotics, production of lincomycin by different *Streptomyces* species was marked by efforts to increase production yields and optimize the product by for instance producing the desirable lincomycin A without the concomitant production of lincomycin B [4–11] *i.e.* 4′-depropyl-4-ethyllincomycin, which appears often as a minority component in production cultures. Its antibiotic activity is a mere 25% as compared with lincomycin A. Attempts at minimizing the amount of lincomycin B during the fermentation [12–14] included the addition of propylproline, a precursor of propylhygric acid in lincomycin A, to the cultivation medium. The mechanism by which the addition of propylproline or L-tyrosine and similar compounds influences the biosynthesis of lincomycin has not yet been clarified but N-demethyllincomycin synthetase, the enzyme catalyzing the linkage between the amino acid and sugar moiety of lincomycin, appears to utilize preferentially propylproline. Another naturally occurring antibiotic of this group is celesticetin and desalicetin, the alkaline hydrolysis product of celesticetin [15]. Both compounds are *in vitro* and *in vivo* less effective against a number of microorganisms than lincomycin [16], although celesticetin exhibits a broad antibacterial spectrum, particularly against Gram-positive bacteria. A number of lincomycin derivatives that have been synthesized includes esters, either with organic (from acetate to stearate) or inorganic (phosphoric, carbonic) acids or lincomycin alkyl derivatives (ethers) and salts of

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lincomycin with inorganic acids, *i.e.* hydrochlorides and derivatives of sulfamic acid. Replacement of hydroxyl in the side chain, (*i.e.* C-7 of octose) by chlorine yields clindamycin. Although hundreds of lincomycin derivatives were prepared including those produced by total chemical synthesis, clindamycin is the only drug that has been successfully used in clinical practice. Chemical modification of lincomycin has yielded a number of derivatives with improved properties, whereas biotransformation has so far been less successful. In our previous review article [3], we listed about 20 different derivatives of lincosamides (Table 1 and Fig. 1).

3. Biosynthesis of lincosamides

Studies on the biosynthesis of lincosamides were previously thoroughly described in many reviews, for example in our previous papers [3,17,18]. A basic scheme of lincomycin A biosynthesis starting from two different precursors, a saccharide and an amino acid, is shown in Fig. 2

4. Genetic control of lincosamide biosynthesis

Similarly to other antibiotics [19] produced by actinomycetes, genes coding for lincomycin biosynthesis are clustered together in a single genomic region and closely linked to the corresponding resistance determinants [20].

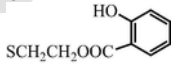
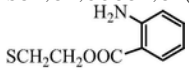
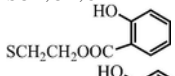
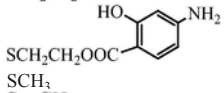
A cosmid was sequenced bearing an insert of 38,217 bp covering the gene cluster and its flanking regions of type strain *S. lincolnensis* ATCC 25466 [21]. Two relatively extensive sequence changes and several hundred point mutations were identified if compared with the previously published sequence of the lincomycin industrial strain *S. lincolnensis* 78–11. Analysis of the cluster-flanking regions revealed its localization within the genome of the ATCC 25466 strain. The cluster-bearing cosmid was integrated into the chromosome of lincomycin non-producing strains *S. coelicolor* CH 999 and *S. coelicolor* M 145. The modified strains heterologously produced Lin but the level dropped to approximately 1–3% of the production in the ATCC 25466 strain.

Čermák and coworkers [22] started comparing lincomycin and celesticetin gene clusters. They presented the first insight into celesticetin biosynthetic gene cluster of *S. caelestis*. The genomic DNA of producing strain was digested, digoxigenin-labeled and hybridized with a set of probes designed according to *S. lincolnensis* gene sequences. Genes with high homology to the lincomycin biosynthetic genes coding for the predicted common parts of the pathway were identified in *S. caelestis*. Genomic DNA of *S. caelestis* treated by a multiple digestion was hybridized with five digoxigenin-labeled probes to construct a rough restriction map. Two consecutive islands formed by the genes with a putative function of the shared saccharide moiety in the biosynthesis revealed an organization similar to the lincomycin biosynthetic gene cluster. The celesticetin cluster was mapped and essential information was obtained for subsequent steps, *i.e.* isolation and sequence analysis of the cluster.

Sasaki and coworkers [23] have recently reported data on the two initial enzymatic steps in the methylthiolincosamide (MTL) biosynthetic pathway leading to the identification of D-*erythro*-D-*gluco*-octose 8-phosphate as a key intermediate. Their experiments demonstrated that this intermediate is formed *via* a transaldol reaction catalyzed by LmbR using D-fructose 6-phosphate or D-sedoheptulose 7-phosphate as the C_3 donor and D-ribose 5-phosphate as the C_5 acceptor. Subsequent 1,2-isomerization catalyzed by LmbN converts the resulting 2-keto C_8-sugar (octulose 8-phosphate) to octose 8-phosphate. These results provide for the first time an *in vitro* evidence revealing the biosynthetic origin of the C_8 backbone of MTL. The authors clearly demonstrated that the proteins lmbR and lmbN are instrumental in this process.

The lmbB2 gene of the lincomycin biosynthetic gene cluster of *S. lincolnensis* ATCC 25466 has been shown to code for an unusual tyrosine hydroxylating enzyme involved in the biosynthetic pathway of this clinically important antibiotic [24]. LmbB2 was expressed in *E. coli*, purified to near homogeneity and shown to convert tyrosine to

Table 1
Structure of lincomycin and related antibiotics.

	Name	R_1	R_2	R_3	R_4	R_5
1	Lincomycin A	SCH_3	CH_3	$CH_2CH_2CH_3$	OH	H
4	Lincomycin B	SCH_3	CH_3	CH_2CH_3	OH	H
5	Lincomycin C	SCH_2CH_3	CH_3	$CH_2CH_2CH_3$	OH	H
7	Lincomycin D	SCH_3	H	$CH_2CH_2CH_3$	OH	H
6	Lincomycin S	SCH_2CH_3	CH_2CH_3	$CH_2CH_2CH_3$	OH	H
8	Lincomycin K	SCH_2CH_3	H	$CH_2CH_2CH_3$	OH	H
10	Lincomycin sulfoxide		CH_3	$CH_2CH_2CH_3$	OH	H
11	1-Demethylthio-1-hydroxylincomycin	OH	CH_3	$CH_2CH_2CH_3$	OH	H
2	Celesticetin A		CH_3	H	OCH_3	H
14	Celesticetin B	$SCH_2CH_2OOCCH_2CH(CH_3)_2$	CH_3	H	OCH_3	H
15	Celesticetin C		CH_3	H	OCH_3	H
16	Celesticetin D	$SCH_2CH_2OOCCH_3$	CH_3	H	OCH_3	H
3	Desalicetin	SCH_2CH_2OH	CH_3	H	OCH_3	H
17	N-Demethylcelesticetin		H	H	OCH_3	H
18	Desalicetinsalicylate		CH_3	H	OCH_3	H
9	Clindamycin	SCH_3	CH_3	$CH_2CH_2CH_3$	H	Cl
12	Clindamycin sulfoxide		CH_3	$CH_2CH_2CH_3$	H	Cl
13	1′-Demethylclindamycin	SCH_3	H	$CH_2CH_2CH_3$	H	Cl

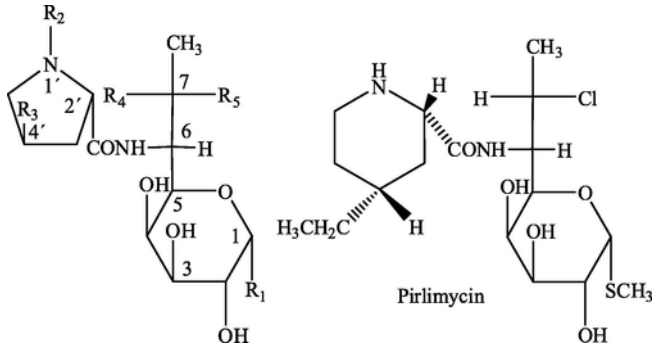


Fig. 1. Structure of lincomycin and related antibiotics.

3,4-dihydroxyphenylalanine (DOPA). In contrast to the well-known tyrosine hydroxylases (EC 1.14.16.2) and tyrosinases (EC 1.14.18.1), LmbB2 was identified as a heme protein. Mass spectrometry and Soret band-excited Raman spectroscopy of LmbB2 showed that LmbB2 contains heme *b* as a prosthetic group. The CO-reduced differential absorption spectra of LmbB2 showed that the coordination of Fe was different from that of cytochrome P450 enzymes. LmbB2 exhibits sequence similarity to Orf13 of the anthramycin biosynthetic gene cluster, which has recently been classified as a heme peroxidase. Tyrosine hydroxylating activity of LmbB2 yielding DOPA in the presence of (6R)-5,6,7,8-tetrahydro-L-biopterin was also observed and tyrosine hydroxylation was confirmed as the first step of the amino acid branch of lincomycin biosynthesis.

Two SAM-dependent *N*-methyltransferases – LmbJ from the biosynthesis of lincomycin and CcbJ from celesticetin biosynthesis – were characterized and compared [25]. Both tested enzymes form multimers and are able to utilize N-demethyllincomycin, the natural substrate of LmbJ, with comparable efficiency. The two methyltransferases were found to have a homooligomeric, probably hexameric, quaternary structure. LmbJ was confirmed to convert N-demethyllincomycin (NDL) into lincomycin. CcbJ is able to catalyze the same reaction with comparable efficiency indicating that its substrate specificity is somewhat relaxed. This finding has biotechnological significance for the design of strains that could produce new lincosamide derivatives with modified amino acid building blocks.

Clinically used lincomycin has been shown to incorporate in its structure 4-propyl-L-proline (PPL), an unusual amino acid, while celesticetin, a less efficient related compound, uses a proteinogenic L-proline [26]. Biochemical characterization, as well as phylogenetic analysis and homology modelling combined with the molecular dynamics simulation were employed for complex comparative analysis of the orthologous protein pair of LmbC and CcbC. The analysis proved that the proteins are stand-alone adenylation domains strictly preferring their own natural substrate, PPL or L-proline. The LmbC substrate binding pocket is adapted to accommodate a rare PPL precursor. When compared with L-proline, several large amino acid residues were replaced by smaller ones opening a channel which allowed the alkyl side chain of PPL to be accommodated. One of the most important differences, that of the residue corresponding to V306 in CcbC which changes to G308 in LmbC, was investigated *in vitro* and *in silico*. Moreover, the substrate binding pocket rearrangement also allowed LmbC to effectively adenylate 4-butyl-L-proline and 4-pentyl-L-proline, substrates with even longer alkyl side chains, producing more potent lincosamides. A shift of LmbC substrate specificity appears to be an integral part of biosynthetic pathway adaptation to the PPL acquisition. A set of genes presumably coding for the PPL biosynthesis is present in the lincomycin but not in the celesticetin cluster; their homologs are found in biosynthetic clusters of some pyrrolobenzodiazepines (PBD) and hormaomycin. Whereas in the PBD and hormaomycin pathways the arising precursors are condensed with another amino acid moiety, the LmbC protein is the first functionally proved part of a unique condensation enzyme connecting PPL to the specialized amino sugar building unit.

In the biosynthesis of lincomycin and celesticetin, the amino acid and amino sugar units have been described to be linked by an amide bond [27]. The respective condensing enzyme lincosamide synthetase (LS) is expected to be an unusual system combining non-ribosomal peptide synthetase (NRPS) components with so far unknown amino sugar related activities. The biosynthetic gene cluster of celesticetin was sequenced and compared to the lincomycin one revealing putative LS coding for ORFs shared in both clusters. Based on a bioassay and production profiles of *S. lincolnensis* strains with individually deleted putative LS coding genes, the proteins LmbC, D, E, F and V were assigned to LS function. Moreover, the newly recognized N-terminal domain of LmbN (LmbN-CP) was also assigned to LS as a NRPS carrier protein (CP). Surprisingly, the homologous CP coding sequence in celesticetin cluster is part of ccbZ gene adjacent to ccbN, the counterpart of lmbN, suggesting gene rearrangement. The *in vitro* test with LmbN-CP, LmbC and the newly identified *S. lincolnensis* phosphopantetheinyl transferase Slp confirmed the cooperation of the previously characterized NRPS A-domain LmbC with a holo-LmbN-CP in activation of a 4-propyl-L-proline precursor of lincomycin. This result completed the functional characterization of LS subunits resembling NRPS initiation module. Two of the four remaining putative LS subunits, LmbE/CcbE and LmbV/CcbV, exhibit low but significant homology to enzymes from the metabolism of mycothiol, the NRPS-independent system processing the amino sugar and amino acid units. The functions of particular LS subunits as well as cooperation of both NRPS-based and NRPS-independent LS blocks have been discussed [27]. The condensing enzyme represents a unique hybrid system with overall composition quite dissimilar to any other known enzyme system. The authors also presented a highly illustrative scheme of the lincomycin and celesticetin gene clusters clearly indicating similarities and differences between the two clusters.

Zhao and coworkers [28] have recently reported that coupling of two bacterial thiols, MSH and EGT, has a constructive role in the biosynthesis of lincomycin A, a sulfur-containing lincosamide (C8 sugar) antibiotic that has for half a century been widely used to treat

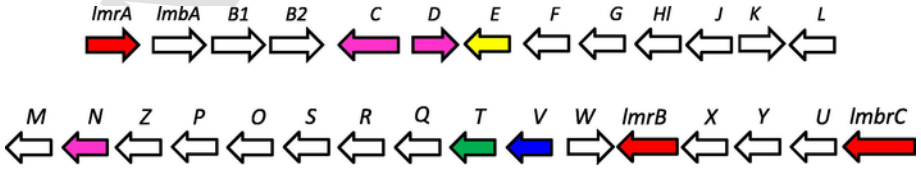


Fig. 2. Biosynthetic gene cluster of lincomycin A. Adapted from Zhao et al. [28]. The *mca* (amidase) encoding homologue *lmbE* is shown in yellow and two GTase-encoding genes *lmbV* and *lmbT* are shown in blue and green, respectively. The genes responsible for PPL incorporation (*lmbC*, *lmbD* and *lmbN*) are shown in pink. Antibiotic resistance coding genes *lmrA*, *lmrB* and *lmrC* are shown in red.

Gram-positive bacterial infections. EGT acts as a carrier to template the molecular assembly, and MSH is the sulfur donor for lincomycin maturation after thiol exchange. The authors showed that these thiols function through two unusual S-glycosylations that program lincosamide transfer, activation and modification, providing the first paradigm for EGT-associated biochemical processes for the poorly understood MSH-dependent biotransformations, a newly described model that is potentially common in the incorporation of sulfur that is essential for life and ubiquitous in living systems. The authors also designed a highly illustrative model of the biosynthetic gene cluster of lincomycin A.

Structurally different and functionally diverse natural compounds – antitumor agents pyrrolo[1,4]benzodiazepines, bacterial hormone hormaomycin, and lincosamide antibiotic lincomycin have been demonstrated to share a common building unit, 4-alkyl-L-proline derivative (APD) [29]. APDs arise from L-tyrosine through a special biosynthetic pathway. The generally accepted scheme, however, did not comply with current state of knowledge. Based on gene inactivation experiments and *in vitro* functional tests with recombinant enzymes, the authors designed a new APD biosynthetic scheme for the model of lincomycin biosynthesis. In the new scheme at least one characteristic in each of five final biosynthetic steps – the order of reactions, assignment of enzymes and/or reaction mechanisms – has been changed. It was first demonstrated that LmbW methylates a different substrate than previously assumed. Second, the authors proposed a unique reaction mechanism for the next step, in which a putative γ-glutamyltransferase LmbA indirectly cleaves off the oxalyl residue by transient attachment of glutamate to LmbW product. This unprecedented mechanism would represent the first example of the C–C bond cleavage catalyzed by a γ-glutamyltransferase, *i.e*., an enzyme that appears unsuitable for such activity. Finally, the inactivation experiments showed that LmbX is an isomerase indicating that it transforms its substrate into a compound suitable for reduction by LmbY, thereby facilitating its subsequent complete conversion to APD 4-propyl-L-proline. Elucidation of the APD biosynthesis has long time resisted clarification mainly due to the apparent absence of relevant C–C bond cleaving enzymatic activity. The above proposal aims to unblock this situation not only for lincomycin biosynthesis, but generally for all above mentioned groups of bioactive natural products with biotechnological potential. The authors also presented a model of biosynthetic gene cluster governing the biosynthesis of lincomycin (Fig 3).

5. Mechanism of action

Idealized diagram of the effect of lincosamides is shown in Fig. 4. Lincosamides belong to antibiotics blocking microbial protein synthesis that includes a large number of steps from activation of amino acid monomers by aminoacyl-tRNA synthesis to many steps of chain initiation, elongation, and chain termination of the grown polypeptides on the ribosome. Antibiotics interrupt the timing and specificity of these steps, and such disruptions decelerate the growth or are lethal to the microorganism. The original lincosamide, lincomycin, has been superseded by clindamycin, which exhibits improved antibacterial activity. Lincomycin and clindamycin possess good antistaphylococcal and antistreptococcal activity. *In vitro* studies demonstrated that these antibiotics even in low concentrations also reduce toxin release by producer strains. Clindamycin has also been proved to be therapeutically useful in the treatment of infections caused by *Bacteroides fragilis* and some other anaerobes. *Pseudomonas aeruginosa* is outside the spectrum of activity. Clindamycin is active against parasitic protozoa and has been used in toxoplasmosis, malaria and babesiosis.

The molecular mechanism by which clindamycin inhibits ribosomal protein synthesis seems to be associated with the fact that clindamycin's three-dimensional structure closely resembles L-Pro-Met and the D-ribosyl ring of adenosine occurring near one another at the 3′-ends of L-Pro-Met-tRNA and deacylated-tRNA for a brief interval following the formation of a peptide bond between L-Pro-tRNA and L-Met-tRNA. Clindamycin and other lincosamides thus may act as structural analogs of the 3′-ends of L-Pro-Met-tRNA and deacylated-tRNA during the initial phase of pretranslocation in the peptide elongation cycle. Although the chemical structure of macrolides (*e.g.* erythromycin), lincosamides (*e.g.* lincomycin, clindamycin and celesticetin) and streptogramins is very different, their mechanism of action is similar. Erythromycin binding with the 23S rRNA blocks polypeptide translation, resulting in a premature release of peptidyl-tRNA intermediates by blocking the approach to the elongating peptide's exit tunnel [30]. Although the macrolides in general do not directly block the peptide bond-forming step at the peptidyltransferase center of the 50S subunits, it is known that they compete with lincosamide antibiotics that are direct peptidyltransferase inhibitors. Lincomycin and clindamycin share a common mechanism of action on sensitive microorganisms and also exhibit a similar antibacterial spectrum. Nevertheless, slight differences in their antimicrobial activity exist as clindamycin also affects some protozoa, *e.g*. *Toxoplasma gondii*, *Plasmodium falciparum* and *Pneumocystis carinii*. Clindamycin inhibits protein synthesis and acts specifically on the 50S subunit of the bacterial ribosome by affecting the process of peptide chain initiation, and may also stimulate dissociation of peptidyl-tRNA from ribosomes.

In a pioneering work Tenson and coworkers [31] studied the mechanisms of action of four macrolides (erythromycin, josamycin, spiramycin and telithromycin), one lincosamide (clindamycin) and one streptogramin B (pristinamycin IA). All these MLS drugs cause dissociation of peptidyl-tRNA from the ribosome. Josamycin, spiramycin and clindamycin, that extend to the peptidyl transferase center, cause dissociation of peptidyl-tRNAs containing two, three or four amino acid residues. Erythromycin, which does not reach the peptidyl transferase center, induces dissociation of peptidyl-tRNAs containing six, seven or eight amino acid residues. Pristinamycin IA causes dissociation of peptidyl-tRNAs with six amino acid residues and telithromycin allows polymerization of nine or ten amino acid residues before peptidyl-tRNA dissociates. These data suggest a common mode of action for all MLS antibiotics, which is modulated by the space available between the peptidyl transferase center and the drug.

In an outstanding review article Gaillard and coworkers [32] concentrated on malaria, a parasite vector-borne disease, one of the biggest health threats in tropical regions despite the availability of malaria chemoprophylaxis. The emergence and rapid extension of *Plasmodium falciparum* resistance to various anti-malarial drugs have gradually limited the potential malaria therapeutics available to clinicians. In this context, macrolides and associated antibiotics based on a similar mechanism of action like lincosamides constitute an interesting alternative in the treatment of malaria. Recent studies have examined the effects of azithromycin and clindamycin on isolates from different continents. Azithromycin and clindamycin are effective and well tolerated in the treatment of uncomplicated malaria in combination with quinine. The review [33] assessed the roles of macrolides and lincosamides in the prophylaxis and treatment of malaria and the mechanism of action of lincosamides on other microorganisms.

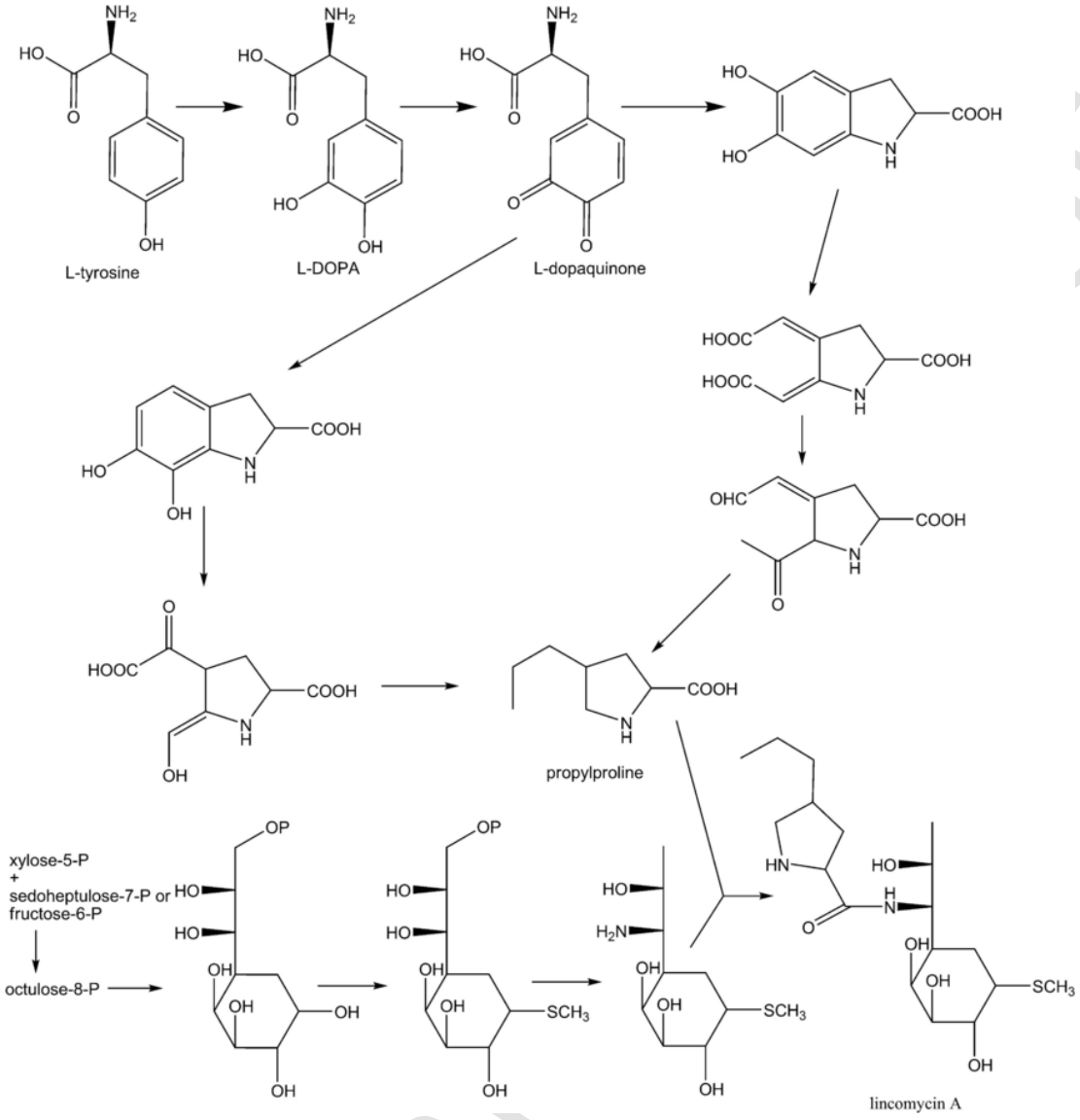


Fig. 3. A basic scheme of lincomycin A biosynthesis starting from two different precursors, a saccharide and an amino acid.

6. Resistance against lincosamide antibiotics

Antibiotic-resistant bacteria that are difficult or impossible to treat are becoming increasingly common and are causing a global health crisis. Antibiotic resistance is encoded by several genes, many of which can transfer between bacteria. New resistance mechanisms are constantly being described, and new genes and vectors of transmission are identified on a regular basis. The emergence of pathogenic bacteria resistant to many commonly used antibiotics has led to a renewed interest in understanding the biochemical basis of antibiotic action and resistance. In general, basic mechanisms of antibiotic resistance include microbial cell impermeability, target site modification, enzymatic modification or destruction of the antibiotic and increased efflux. The main type of resistance to lincomycin and clindamycin is the so-called MLS_B resistance which renders sensitive microorganism resistant to macrolides, lincosamides and streptogramin B. It is a monomethylation or dimethylation of the N6 exocyclic amino group of A_{2058} by specific ribosome methylation modification enzymes. This type of resistance is associated with genes encoding methyltransferases modifying the common target site of macrolides and lincosamides, *i.e*. 23S ribosomal RNA (*e.g*. genes ermA and ermC). A specific gene was also described whose protein product modifies and thus inactivates lincosamide antibiotics (linA). Lincosamide resistant *Streptococcus pneumoniae* carries mutations in the 23S rDNA with substitutions at A2058, A2059, or C2611 and in L4 or L22 ribosomal protein genes. 50S Ribosomal mutations are the least frequent mechanism of *S. pneumonie* resistance [34]. Kehrenberg and coworkers [35] and Long and coworkers [36] reported on a new mechanism for chloramphenicol, florfenicol and clindamycin resistance, *viz*. methylation of 23S ribosomal RNA at A2503 by the *cfr* gene product from *Staphylococcus sciuri*, *S. aureus* and *E. coli*. The results show that *Cfr* is an RNA methyltransferase that targets nucleotide A2503 and causes resistance to chloramphenicol, forfenicol and clindamycin by inhibiting ribose methylation at nucleotide C2498. As described by Rich and coworkers [37], resistance to clindamycin in methicillin-resistant *Staphylococcus aureus* may be due to modification of the ribosomal target site. Resistance due to this

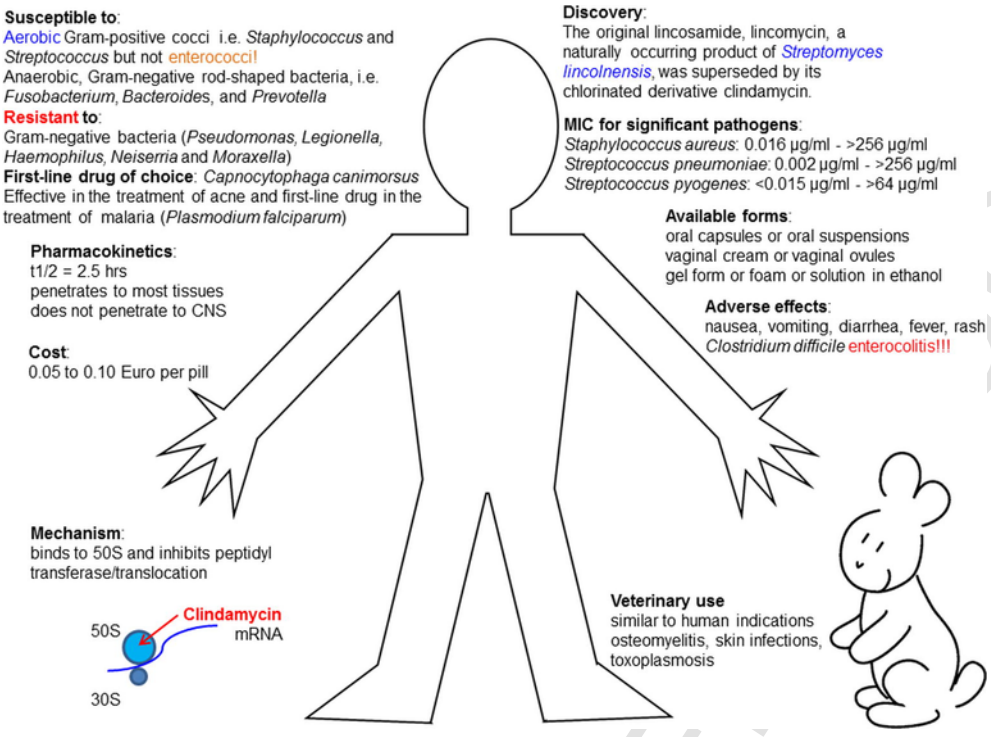


Fig. 4. Idealized diagram of the pharmacological effect of lincosamides.

mechanism is encoded by the *errs* genes, which may be either constitutively or inducibly expressed. Inducible resistance is not usually revealed by routine laboratory susceptibility testing but can be detected by a relatively simple double disc agar diffusion test. Since staphylococci can develop resistance to clindamycin even during treatment when inducible resistance mechanisms are present, routine screening for inducible resistance is recommended.

Nir-Paz and coworkers [38] evaluated the susceptibility to macrolide, lincosamide and tetracycline of invasive *Streptococcus pyogenes*. In their study they found that group A β-hemolytic streptococcus strains in Israel are extremely rarely resistant to clindamycin, permitting its use in the treatment of infections caused by them.

Champney and Tober [39] investigated specific inhibition of 50S ribosomal subunit formation in *Staphylococcus aureus* by a 16-membered macrolide, lincosamide, and streptogramin B antibiotics. The lincosamides lincomycin and clindamycin contain linked 5- and 6-membered rings along with an essential sulfur group and interact with the 50S particle, similarly to macrolides.

In an extensive review article concerning macrolide-resistant *Streptococcus pyogenes* [40] the authors showed that macrolides are important alternatives for allergic patients and lincosamides are recommended together with β-lactams in invasive infections. *Streptococcus pyogenes* exhibits macrolide resistance based on active efflux or target modification, the latter conferring cross resistance to lincosamides and streptogramin B. In a highly detailed table the authors showed that resistance and the associated phenotypes are highly variable across countries. Surprisingly, an overall decreasing trend in resistance can be observed in Europe, which is certainly a comforting situation.

In an interesting and extensive review Colabroy [41] summarized the facts about metabolic pathways for the production of lincomycin, hormaomycin and the antitumor pyrrolobenzodiazepins (VPCA). Regions of biosynthetic gene clusters specifying the biosynthesis of anthramycin, sibiromycin, tomaymycin, lincomycin and hormaomycin have been shown, nicely illustrating common features of the clusters in question. The author proposed that further studies of the structures of L-tyrosine hydroxylase and L-DOPA dioxygenase should reveal how these enzymes accomplish their biosynthetic roles.

Leclercq [42] defined basic mechanisms by which bacteria resist antibiotics as including: (1) target-site modification by methylation or mutation that prevents the binding of the antibiotic to its ribosomal target, (2) efflux of the antibiotic and, (3) drug inactivation. According to the author "modification of the ribosomal target confers broad-spectrum resistance to macrolides and lincosamides, whereas efflux and inactivation affect only some of these molecules"

One of the papers from our laboratory [43] reported that a high occurrence of the non-macrolide-streptogramin B resistance genes *msrA* (53%) and *linA*/*linA′* (30%) could be detected among 98 methicillin-resistant coagulase-negative staphylococci additionally resistant to macrolides and/or lincosamides. The gene *msrA* was found to predominate in *Staphylococcus haemolyticus* (43 of 62 isolates). In *Staphylococcus epidermidis*, it was found in 7 of 27 isolates. The study proposed a new mechanism of resistance to lincosamides in 10 genetically related isolates of *S. haemolyticus* in the absence of *ermA*, *ermC*, *msrA*, and *linA*/*linA′*.

An outstanding paper from Gerry Wright's laboratory [44] showed that lincosamide-resistant microorganisms have predictably emerged with antibiotic modification as one of their major resistance strategies. Inactivating enzymes LinB/A were presumed to catalyze adenylylation of the drug. In order to elucidate their mechanistic and structural properties two X-ray structures of LinB: ternary substrate- and binary product-bound complexes were determined. Structural and kinetic characterization of LinB, mutagenesis, solvent isotope effect, and product inhibition studies were consistent with a mechanism involving direct in-line nucleotidyl transfer. According to the authors LinB can be classified as a member of a nucleotidyltransferase family, along with nucleotide polymerases and aminoglycoside nucleotidyltransferases, and this relationship offers a further support for

the LinB mechanism. It appears that the LinB structure provides an evolutionary link to ancient nucleotide polymerases suggesting that these are proto-resistance elements from which drug resistance can evolve. Biochemical studies performed by the authors showed that LinB readily inactivates clindamycin *in vitro* with the turnover of 0.3 s^{-1}, and an NMR analysis showed that the 3′-OH of clindamycin is the site of adenylylation.

A new variant of the streptogramin A resistance gene, vga(A)LC, was found in clinical isolates of *S. haemolyticus* resistant to lincomycin and clindamycin but susceptible to erythromycin and in which no relevant lincosamide resistance gene was detected [45]. The gene vga(A)LC, differing from the gene vga(A) at the protein level by seven amino acid substitutions, was present exclusively in *S. haemolyticus* strains resistant to both lincosamides and streptogramin A (LS(A) phenotype). Antibiotic resistance profiles of the ATP-binding cassette (ABC) proteins Vga(A)(LC) and Vga(A) in the antibiotic-susceptible host *S. aureus* RN4220 were compared. It was shown that Vga(A)LC conferred resistance to both lincosamides and streptogramin A, while Vga(A) conferred significant resistance to streptogramin A only. Detailed analysis of the seven amino acid substitutions, distinguishing the two related ABC proteins with different substrate specificities, identified the substrate-recognizing site: four clustered substitutions (L212S, G219V, A220T, and G226S) in the spacer between the two ATP-binding cassettes altered the substrate specificity and constituted the lincosamide-streptogramin A resistance phenotype. A transport experiment with radiolabeled lincomycin demonstrated that the mechanism of lincosamide resistance in *S. haemolyticus* was identical to that of the reported macrolide-streptogramin B resistance conferred by Msr(A).

Szczuka and coworkers [46] studied coagulase-negative staphylococci (CoNS) that are the most frequently isolated bacteria from the blood and the predominant cause of nosocomial infections. According to the authors, macrolides, lincosamides and streptogramin B (MLS_B) antibiotics, especially erythromycin and clindamycin, are important therapeutic agents in the treatment of methicillin-resistant staphylococcal infections. Among CoNS, *Staphylococcus hominis* represents the third most common organism. In spite of its clinical significance, very little is known about its mechanisms of resistance to antibiotics, especially MLS_B. The authors studied 55 *S. hominis* isolates from the blood and the surgical wounds of hospitalized patients. The *erm*(C) gene was predominant in erythromycin-resistant *S. hominis* isolates. The methylase genes, *erm*(A) and *erm*(B), were present in 15 and 25% of clinical isolates, respectively. A combination of various erythromycin resistance methylase (*erm*) genes was detected in 15% *S. hominis* isolates. The efflux gene *msr*(A) was detected in 18% of isolates, alone in four isolates, and in different combinations in a further six. The *lnu*(A) gene, responsible for enzymatic inactivation of lincosamides, was carried by 31% of the isolates. No erythromycin resistance that could not be attributed to the genes *erm*(A), *erm*(B), *erm*(C) and *msr*(A) was detected. Of *S. hominis* isolates, 75 and 84%, respectively, were erythromycin resistant and clindamycin susceptible. Altogether 68% of erythromycin-resistant *S. hominis* isolates, showed an inducible MLS_B phenotype. Four isolates harboring the *msr*(A) genes alone displayed the MS_B phenotype. These studies indicated that resistance to MLS_B in *S. hominis* is mostly based on the ribosomal target modification mechanism mediated by *erm* genes, mainly the *erm*(C), and enzymatic drug inactivation mediated by *lnu*(A).

Roberts [47] reviewed data on environmental macrolide-lincosamide-streptogramin and tetracycline resistant bacteria. In the table summarizing MLS resistance genes the author included 34 rRNA methylase resistance genes, 17 efflux resistance genes, and 19 genes governing inactivating enzymes 2 esterases, 2 lysases, 11 transferases and 4 phosphorylases.

7. Intrinsic and acquired resistance

Bacteria can be intrinsically resistant to certain antibiotics but can also acquire resistance to antibiotics *via* mutations in chromosomal genes and by horizontal gene transfer. The intrinsic resistance of a bacterial species to a particular antibiotic is the ability to resist the action of that antibiotic as a result of inherent structural or functional characteristics.

In general, Gram-positive microorganisms are sensitive to lincomycin and clindamycin. *Bacillus anthracis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, *Streptococcus pyogenes* and *Streptococcus viridans* can serve as examples. On the other hand, Gram-negative microorganisms are generally resistant to lincomycin and clindamycin. *E. coli*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria gonorhoeae*, *Neisseria meningitis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, and *Salmonella* sp. are typical representatives. Among anaerobic bacteria *Clostridium tetani* and *Clostridium perfringens* are sensitive to clindamycin, but some *C. perfringens* strains and strains of *C. sporogenes*, *C. tercium*, *C. bifermentans*, *C. novyi*, *C. ramosum*, and *C. sordelli* may be clindamycin-resistant. Clostridium difficile is now established as a major nosocomial pathogen. *C. difficile* infection is observed almost exclusively as a complication of antibiotic therapy and is particularly observed with clindamycin and third generation cephalosporins [48]. However, the data of some recent studies, *e.g.* those by Forster [49], do not seem so obvious.

As pointed out by Blair and coworkers [50] in their excellent review, antibiotics underpin modern medicine; their use has reduced childhood mortality and increased life expectancy, and they are crucial for invasive surgery and treatments such as chemotherapy. However, the number of infections caused by multidrug-resistant bacteria is increasing globally, and the increase in untreatable infections is becoming a reality.

According to the authors antibiotic resistance includes: 1. Prevention of access to target including reduced permeability and increased efflux. 2. Changes in antibiotic targets by mutation. 3. Modification and protection of targets. 4. Direct modification of antibiotics including inactivation of antibiotics by hydrolysis and by transfer of a chemical group.

8. Biological activity and applications

When usual doses are applied, both lincomycin and clindamycin are bacteriostatic. At higher concentrations their effect may be even bactericidal. However, the onset of the bactericidal effect is delayed and is less complete than that of *e.g.* β-lactams. It increases generally in parallel with increasing concentration of the antibiotic and, even in this respect, clindamycin is more effective. On the other hand, the main advantage of lincomycin is that it can be applied in a substantially wider concentration range of clinical therapeutically effective doses [51].

Macrolides and lincosamides are first-choice bacteriostatic antibiotics used in veterinary microbiology. The main antibiotics of this class are erythromycin, lincomycin, clindamycin and tylosin. Their spectrum of action is commonly associated with skin infections, including staphylococcal infections. Their main disadvantage is the rapid development of bacterial resistance and occasional gastrointestinal upset [52].

9. Conclusions and future prospects

Lincomycin and clindamycin are clinically important antibiotics. The semisynthetic lincosamide derivative clindamycin is one of the 20 most important antibiotic compounds.

Macrolide, lincosamide, and streptogramin (MLS) antibiotics are chemically distinct but have a similar mode of action. They have a narrow spectrum of activity that includes Gram-positive cocci (in particular, staphylococci, streptococci, and enterococci) and bacilli and Gram-negative cocci. These drugs, especially clindamycin, are also potent against anaerobic bacteria. Gram-negative bacilli are usually resistant to MLS antibiotics, but certain enteric bacilli and *Haemophilus* spp. are susceptible to azithromycin *in vitro*.

Lincomycin and clindamycin also exhibit good antistaphylococcal and antistreptococcal activity. *In vitro* studies also demonstrated reduced toxin release by producer strains even in the presence of low concentrations of clindamycin. Clindamycin has also proved efficient in the treatment of infections caused by *Bacteroides fragilis* and some other anaerobes. Enterobacteria and *Pseudomonas aeruginosa* are not affected. Clindamycin and some of its derivatives are used in the treatment of malaria caused by *Plasmodium falciparum* and in the treatment of toxoplasmosis and babesiosis. Lincosamides are used in the prevention of intraabdominal infections after surgery, stomatological infections, anaerobic sepsis and especially of bone and articular infections.

Chemical modification of lincomycin yielded a number of derivatives with improved properties, whereas biotransformation has so far been less successful. However, with the current knowledge of gene clusters governing the biosynthesis of lincomycin (also briefly discussed in this review), we can easily imagine production of new derivatives of lincosamides. The analysis of genes specifying biosynthesis of antibiotics can be used for the development of new derivatives of antibiotics including lincosamides. Actinomycetes are amazingly prolific in the number of antibiotics they produce [51]. According to the Microbial Genome Database for Comparative Analysis a total of 18 *Streptomyces* genomes have been sequenced, including *Streptomyces albus*, *S. collinus*, *S. davawensis*, *S. flavogriseus*, *S. fulvissimus* and different species of *S. hygroscopicus*, *S. venezuelae* and *S. violaceusniger*. It was proposed that this knowledge can certainly be utilized in the search for new antibiotics [53] including the search for new lincosamide compounds. In this respect, it appears that lincosamides are not only useful antibiotics, especially for the treatment of anaerobic and protozoal infections, but that they also may serve as a model for the future development of new derivatives of antibiotics by means of genetic engineering [42]. One of the possible ways of coping with a permanently increasing antibiotic resistance could also be the revival of old antibiotics as proposed in an excellent review article by Cassir and coworkers [54].

Spellberg and coworkers [55] in a highly interesting review referred to the annual report on global risks by the World Economic Forum (WEF) concluding that "the greatest risk to human health comes in the form of antibiotic-resistant bacteria" and that "we live in a bacterial world where we will never be able to stay ahead of the mutation curve". They indicated several important points: 1. Collapse of the antibiotic research and development of new antibiotics. 2. Resistance is primarily the result of bacterial adaptation to billions of years of antibiotic exposure. 3. After billions of years of evolution, microbes have apparently invented antibiotics against every biochemical target that can be attacked.

The authors also emphasized that resistance was found even to synthetic antibiotics that did not exist on Earth until the 20th century. The danger of a "general antibiotic resistance" thus seems to be real and cannot be underestimated. For years antibiotics have provided treatment for a number of diseases caused by microorganisms. However, as mentioned many times previously, the number of microorganisms that have adapted to the current antibiotics has been increasing dramatically. Many challenges face the discovery and development of new antibiotics, making it for them difficult to reach the market, especially since many pharmaceutical companies have stopped searching for antibiotics [56].

In 2010 Davies and Davies concluded [57] that we should renew a concerted attack on antibiotic resistance, otherwise "the preantibiotic era awaits our descendants".

We (J.S. and T.R.) are deeply convinced that the search for new antibiotics, including lincosamides, should continue unabated.

Conflict of interest

The authors declare that there are no conflicts of interest regarding this work.

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