Lincosamides: Chemical Structure, Biosynthesis, Mechanism of Action, Resistance, and Applications

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I. Introduction

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 semi-synthetic derivatives of lincomycin have been prepared. Of these, only the chlorinated derivative clindamycin is highly biologically active and is applied practically. Natural lincosamides are produced by several *Streptomyces* species, mainly by *Streptomyces lincolnensis, S. roseolus*, and *S. caelestis* and by *Micromonospora halophytica*. Their mechanism of action is via inhibition of protein synthesis in sensitive microorganisms. Lincosamides have an unusual antimicrobial spectrum, being active against only Gram-positive and not Gram-negative aerobic bacteria but widely and potently active against anaerobic bacteria and some protozoa.

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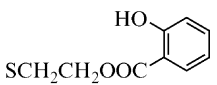
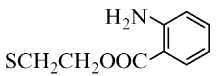
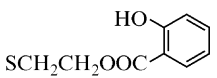
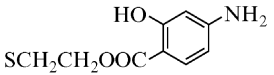
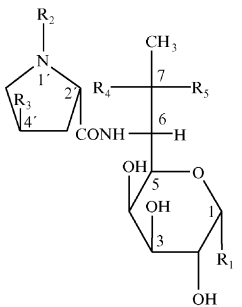
TABLE I

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	$S(=O)-CH_3$	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



The major route of resistance to lincosamides is modification of the 23S rRNA in the 50S ribosomal subunit, similarly to resistance against macrolides and streptogramin B (MLS_b resistance). Clindamycin is the main lincosamide antibiotic that is applied in clinical practice.

II. Chemical Structure of Lincosamides and Cultivation of Production Strains

The chemical structure of lincomycin was investigated by Hoeksema *et al.* (1964), employing both classical chemical degradation and nuclear magnetic resonance. These studies resulted in the chemical structure of lincomycin (1), (Table I). It 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 (methylthiolincosamide). Details of the chemical characterization and nuclear magnetic resonance studies of lincomycin and its degradation products were subsequently described (Herr and Slomp, 1967; Magerlein, 1971; Schroeder *et al.*, 1967; Slomp and MacKellar, 1967). The electron-impact mass spectrum and the chemical-ionization mass spectrum of lincomycin were reported later (Horton *et al.*, 1974; Kagan and Grostic, 1972).

Lincomycin belongs to a novel class of antibiotics characterized by an alkyl 6-amino-6,8-dideoxy-1-thio-D-*erythro*-α-D-galacto-octopyranoside joined with a proline moiety by an amide linkage. The availability of this structure presented an excellent opportunity for chemical and microbiological modification of lincomycin.

Before a review of the other naturally occurring members of the lincomycin family of antibiotics, processes used for the cultivation of producing strains (Table II) and methods for separation of lincomycin A will be described.

The first patent describing lincomycin production was registered by Upjohn (Bergy *et al.*, 1963). *S. lincolnensis* var. *lincolnensis* was cultivated in a 380-liter fermentor at 28 °C for 5 days, and 210 liters of cultivation broth were harvested to give approximately 105 g of dried lincomycin A at a purity of 232 biounits/mg.

The actinomycete *S. vellosus* var. *vellosus* isolated from Arizona soil was used according to Bergy *et al.* (1981) for the production of lincomycin A without the concomitant production of lincomycin B (see subsequent discussion).

The novel actinomycete described in the patent of Argoudelis *et al.* (1972) produced only lincomycin A. *S. espinosus* produced 50 μg/ml of the desirable lincomycin A during a 4-h cultivation at 28 °C in

TABLE II

STRAINS PRODUCING LINCOMYCIN GROUP OF ANTIBIOTICS

Organisms	Literature
S. lincolnensis 78–11	Peschke *et al.* (1995), Zhang *et al.* (1992)
S. lincolnensis NRRL 2936	Bergy *et al.* (1963), Brahme *et al.* (1984a), Peschke *et al.* (1995)
S. lincolnensis RIA 1246	Neusser *et al.* (1998)
S. lincolnensis UC 5124	Chung and Crose (1990)
S. espinosus NRRL 5729	Peschke *et al.* (1995)
S. espinosus NRRL 3890	Peschke *et al.* (1995)
S. espinosus	Argoudelis *et al.* (1972)
S. espinosus	Reusser and Argoudelis (1974)
S. pseudogriseolus NRRL 3985	Peschke *et al.* (1995)
S. variabilis var. *liniabilis*	Argoudelis and Coats (1974)
S. vellosus NRRL 8037	Bergy *et al.* (1963), Peschke *et al.* (1995)

shaken flasks by using a classical medium. According to the U.S. patent (Argoudelis and Coats, 1973), *S. pseudogriseolus* var. *linmyceticus* cultivated for 2 days at 28 °C produces ~22 μg/ml of lincomycin. Another patent on production of lincomycin A only (Argoudelis and Coats, 1974), without contamination with lincomycin B, was registered by The Upjohn Company. *Streptomyces variabilis* var. *liniabilis* produces 26 μg/ml of lincomycin A after the 120-h cultivation at 28 °C in shaken 500-ml Erlenmeyer flasks.

The strain *S. lincolnensis* used by Zhang (1993) is a phage-resistant, lincomycin-overproducing mutant synthesizing approximately 2.5 g of lincomycin A per liter.

In the last 5 years, Chinese investigators have become interested in the cultivation of lincomycin-producing strains, especially in the isolation of lincomycin with a simultaneous decrease or complete removal of the undesirable lincomycin B (Dong and Dong, 2001; Li *et al.*, 2001; Wang and Qi, 1999, 2000, 2001).

In addition to lincomycin, a very closely related antibiotic was isolated from fermentations of *Streptomyces lincolnensis* var. *lincolnensis*. Structural studies indicated this compound to be lincomycin B (i.e., 4′-depropyl-4-ethyllincomycin (2). It had already been

described in 1965 as a minority component in *S. lincolnensis* cultures. It exhibits only a 25% antibiotic activity as compared with lincomycin A. As shown in Table I, lincomycin B contains one fewer methylene group in the side chain of propylhygric acid. Several patents (Reusser and Argoudelis, 1974; Visser, 1972; Witz, 1972) were published in which procedures were described to minimize the amount of lincomycin B during the fermentation. The addition of propylproline to the cultivation medium was found to decrease the amount of lincomycin B. It was assumed that propylproline is a likely precursor of propylhygric acid in lincomycin A, whereas ethylproline is a tentative precursor of ethylhygric acid of lincomycin B. Aromatic amino acids, primarily L-tyrosine, L-dihydroxyphenylalanine, and other compounds similar to tyrosine, are by *S. lincolnensis* incorporated into lincomycin A. It was found that L-tyrosine or L-dihydroxyphenylalanine added to the culture medium induces accumulation of propylproline and also, although to a lesser extent, that of ethylproline. The mechanism by which the addition of propylproline or L-tyrosine and similar compounds influences the biosynthesis of lincomycin has not yet been clarified; however, the results obtained so far indicate that N-demethyllincomycin synthetase, the enzyme catalyzing the linkage between the amino acid and sugar moiety of lincomycin, preferentially utilizes propylproline.

Lincomycin A was the first member of its family whose structure had been completely elucidated; however; celesticetin, a related antibiotic, was reported earlier in the culture broth of *Streptomyces caelestis*, a new actinomycete species. This antibiotic was also isolated by Hoeksema *et al.* (1955). Employing some of the techniques used in the structural studies on lincomycin, Hoeksema (1968) assigned structure 3 to celesticetin. Desalicetin, the alkaline hydrolysis product of celesticetin, was shown to possess structure 4. Celesticetin and desalicetin proved to be less effective than lincomycin against a number of microorganisms, both *in vitro* and *in vivo* (Mason and Lewis, 1964). Celesticetin exhibits a broad antibacterial spectrum, particularly against Gram-positive bacteria. Other new antibiotics, *viz.* N-demethyl-7-O-demethylcelesticetin and N-demethylcelesticetin, were found to be produced in cultures of a strain of *S. caelestis*.

The introduction of various chemicals to the fermentation of *S. lincolnensis* var. *lincolnensis* was found to induce the formation of lincomycin-related antibiotics. Thus the addition of DL-ethionine led to the formation of 1-demethylthio-1-ethylthiolincomycin (5) (Argoudelis and Mason, 1965) and 1-demethyl-1-demethylthio-1′-

ethyl-1-ethylthiolincomycin (6) (Argoudelis *et al.*, 1970); methylthiolincosaminide induced formation of 1′-demethyllincomycin (7); the addition of ethyl thiolinconsaminide yielded 1′-demethyl-1-demethylthio-1-ethylthiolincomycin (8). Sulfonamides, sulfanilamide in particular, inhibit N-methylation rather selectively, resulting in the production of 1′-demethyllincomycin (7).

Chemical modification (Argoudelis and Stroman, 1984; Birkenmeyer, 1981, 1982a,b; Patt *et al.*, 1984) of lincomycin derivatives consisted mainly in substitution of hydrogen atoms bound to a heteroatom. A number of lincomycin esters, with either organic (from acetate to stearate) or inorganic (phosphoric, carbonic) acids or of lincomycin alkyl derivatives (ethers), were prepared. Substitutions were performed primarily in positions 2, 3, 4, and 7. Salts of lincomycin with inorganic acids (i.e., hydrochlorides and derivatives of sulfamic acid) were also synthesized. Replacement of hydroxyl in the side chain (i.e., C-7 of octose) is another modification of lincomycin. The commercially used derivative with the generic name clindamycin (9) was produced by introduction of chlorine. Additional thio-analogs, of which, for example, the C-1 β-anomer exhibits only one tenth of the activity of the natural α-anomer, were synthesized. The changed configuration of 1-methyl-4-proline is also very important. It was demonstrated that the derivative containing the D amino acid exhibits only 50% of the antibacterial activity. Although hundreds of lincomycin derivatives were prepared, among them derivatives produced totally by chemical synthesis, clindamycin is the only drug that has been used successfully in clinical practice. Detailed synthetic procedures leading to numbers of lincomycin derivatives and their biological activity were described by Magerlein (1971).

A new and stereoselective route to the aminoglycoside components of the antibiotics lincomycin and clindamycin has been described. The key step involves diastereoselective introduction of the amino group at C-6 of D-galactose by (3,3)-sigmatropic rearrangements of the corresponding allylic (Z)-trifluoroacetimidate and (Z)- and (E)-allylic thiocyanates. Epoxidation of the resulting trifluoroacetamide with m-chloroperbenzoic acid led to the epoxide with a high threo-selectivity (Gonda *et al.*, 2000). In another paper (Bowden and Stevens, 2000) a novel synthesis of clindamycin from lincomycin by using N-chlorosuccinimide and triphenylphosphine was reported, resulting in high yields and avoiding the use of tetrachloromethane employed in the currently used manufacturing process.

Microbial modification of lincomycin and clindamycin does not lead to the formation of the number and diversity of transformation

products obtained by chemical modification. However, several types of interesting transformations have been reported. Thus, for instance, lincomycin was glycosylated by using jack bean α-mannosidase to produce 7-O-α-D-mannopyranosyl-lincomycin (Weignerova *et al.*, 2001).

The addition of lincomycin to a fermentation of *S. lincolnensis* var. *lincolnensis* resulted in the formation of lincomycin sulfoxide (10). This sulfoxide was also prepared by oxidation of lincomycin hydrochloride with hydrogen peroxide (Pospisil *et al.*, 2001). As compared with lincomycin, it exhibits only a 0.01 activity against *Sarcina lutea*. A lower yield of 1-demethylthio-l-hydroxylincomycin (11) was also isolated from the fermentation broth.

Similarly, clindamycin sulfoxide (12) was the major transformation product when clindamycin was added to fermentations of *S. armentorus*. Trace amounts of sulfoxide were detected in similar fermentations of other *Streptomyces* species, particularly *S. punipalus*. Clindamycin sulfoxide is about equally active as lincomycin against *Sarcina lutea*.

In addition to sulfoxide formation, several species of streptomycetes, including those mentioned above, perform a partial 1-demethylation (Argoudelis *et al.*, 1969). *S. punipalus* was found to demethylate clindamycin (9) to 1′-demethylclindamycin (13), which had previously been prepared by chemical modification. Oxidation of lincomycin with H_2O_2 in alkaline media leads to N-oxides and the conversion of thiomethyl group to sulfoxides and sulfones. NH_4OH favors formation of the *S*-isomer; both *R*- and *S*-isomers of N-oxide form in the presence of NaOH (Pospisil *et al.*, 2004).

Argoudelis and Coats (1969) observed that *S. rochei* grown in a synthetic medium converted lincomycin and lincomycin-related antibiotics to their 3-phosphate esters. Lincomycin 3-phosphate was inactive *in vitro* against several organisms.

In addition to ‘‘classical methods’’ for the analysis of lincomycin and clindamycin, in the last 20 years methods of instrumental analysis based on liquid chromatography (LC) as a separation method and soft ionization techniques of mass spectrometry as an identification method were developed.

Reverse-phase (RP) high-performance liquid chromatography (HPLC) for preparation of lincomycin A was patented (Hofstetter, 1982). This process yielded a highly purified lincomycin A with less than 0.5% lincomycin B. Lincomycin salvage mother liquor, containing about 30% lincomycin B, was used as the starting material for preparation of lincomycin B.

Procedures for the determination of clindamycin in human plasma using HPLC with ultraviolet (UV) detection have been described (Fieger-Büschges *et al.*, 1999; La Follette *et al.*, 1988; Liu *et al.*, 1997). These methods are either not very sensitive or are time-consuming and require laborious sample preparation.

More recently, methods using HPLC coupled with MS detection have been published for the quantification of clindamycin in human plasma. Yu *et al.* (1999) used an LC-MS/MS/ESI method, with acceptable linearity in the 0.05–20 μl/ml range and a quantification limit of 0.050 μg/ml. Martens-Lobenhoffer and Banditt (2001) used an LC-MS/APCI method for both human plasma and bone. The method showed good linearity in the 0.1–4.0 μg/ml range for plasma with a limit of quantification of 0.1 μg/ml.

A method for the quantification of clindamycin in animal plasma by using LC-MS/MS/ESI has also been published (Cherlet *et al.*, 2002). Lincomycin was used as the internal standard. Good linearity was observed within the range of 0–10 μg/ml. The limit of quantification of the method is 50 μg/ml, and the detection limit is 1.3 ng/ml. The method was used for pharmacokinetic studies of clindamycin formulations in dogs.

Lincomycin and related antibiotics were analyzed by an MS/MS/CID technique in bovine milk extract. Lincomycin gave a limit of detection of 0.83 pg on-column (Crellin *et al.*, 2003).

For a further analysis of lincomycin and spectinomycin, an RP ion-pair LC method with a base-deactivated column and pulsed electrochemical detection by means of a gold electrode was used (Szunyog *et al.*, 2002).

Capillary electrophoresis was used for a simultaneous determination of five aminoglycoside antibiotics (netilmicin, tobramycin, lincomycin, kanamycin, and amikacin). Under the optimum separation conditions, the aminoglycoside antibiotics were baseline separated within 20 min and the detection limit was below 6.7 μM for lincomycin (Yang *et al.*, 2001).

HPLC-integrated pulsed amperometric detection was found to be a good primary detection method to complement or replace the absorbance detection method in the case of nonchromophoric sulfur-containing antibiotics and their sulfur-containing impurities and decomposition products (Hanko *et al.*, 2001).

A thin-layer chromatography/densitometric method was developed for the identification and quantitation of oxytetracycline, tiamulin, lincomycin, and spectinomycin in veterinary preparations (Krzek *et al.*, 2000).

III. Lincomycin Biosynthetic Pathway

The lincomycin biosynthetic pathway proceeds via a heterogeneously rooting biphasic pathway, giving rise to propylproline and methylthiolincosamide (Fig. 1). These basic precursors become condensed to N-demethyllincomycin, which is finally methylated to yield lincomycin.

A. Biosynthesis of the Amino Acid Moiety

Tyrosine seems to be the principal precursor of the amino acid moiety. Feeding studies using L-[1-^{14}C]tyrosine and L-[U-^{14}C]tyrosine performed by Witz *et al.* (1971) suggested that seven of the nine carbons in the propylhygric acid come from tyrosine. The other two carbons, *viz.* the N-methyl and terminal aliphatic side chain methyl groups, come from S-adenosylmethionine (Argoudelis *et al.*, 1969; Brahme *et al.*, 1984a,b; Witz *et al.*, 1971).

Biosynthetic experiments with D-($^{13}C_6$) glucose suggest that glucose is converted via glycolysis and the hexose monophosphate shunt to phosphoenolpyruvate and erythrose-4-phosphate, respectively, which are in turn converted via the shikimic acid pathway to tyrosine and then to dihydroxyphenylalanine. The pathway probably continues through the 2,3-extradiol cleavage of the aromatic ring of dihydroxyphenylalanine, followed by condensation to form a pyrrolo ring (Brahme, 1984a). A similar biosynthetic route leading from tyrosine through dihydroxyphenylalanine and pyrrolo ring formation lincomycin probably shares with the pyrrolo[1,4]benzodiazepine antibiotics anthramycin, sibiromycin, and tomaymycin (Hurley, 1980; Hurley *et al.*, 1979). The multistep conversion of dihydroxyphenylalanine to the propylproline remains unknown, but it appears to lead to 1,2,3,6-tetradehydro-propylproline, which was shown to accumulate in mutants lacking a reductase that requires lincomycin cosynthetic factor (Kuo *et al.*, 1992) identified as 7,8-didemethyl-8-hydroxy-5-deazariboflavin (Kuo *et al.*, 1989). Based on this finding, Kuo and coworkers modified the scheme of Brahme *et al.* (1984a,b) and postulated the remaining steps leading to propylproline, as shown in Fig. 1.

B. Biosynthesis of the Sugar Moiety

No intermediates of the aminooctose moiety methylthiolincosamide biosynthesis have so far been identified.

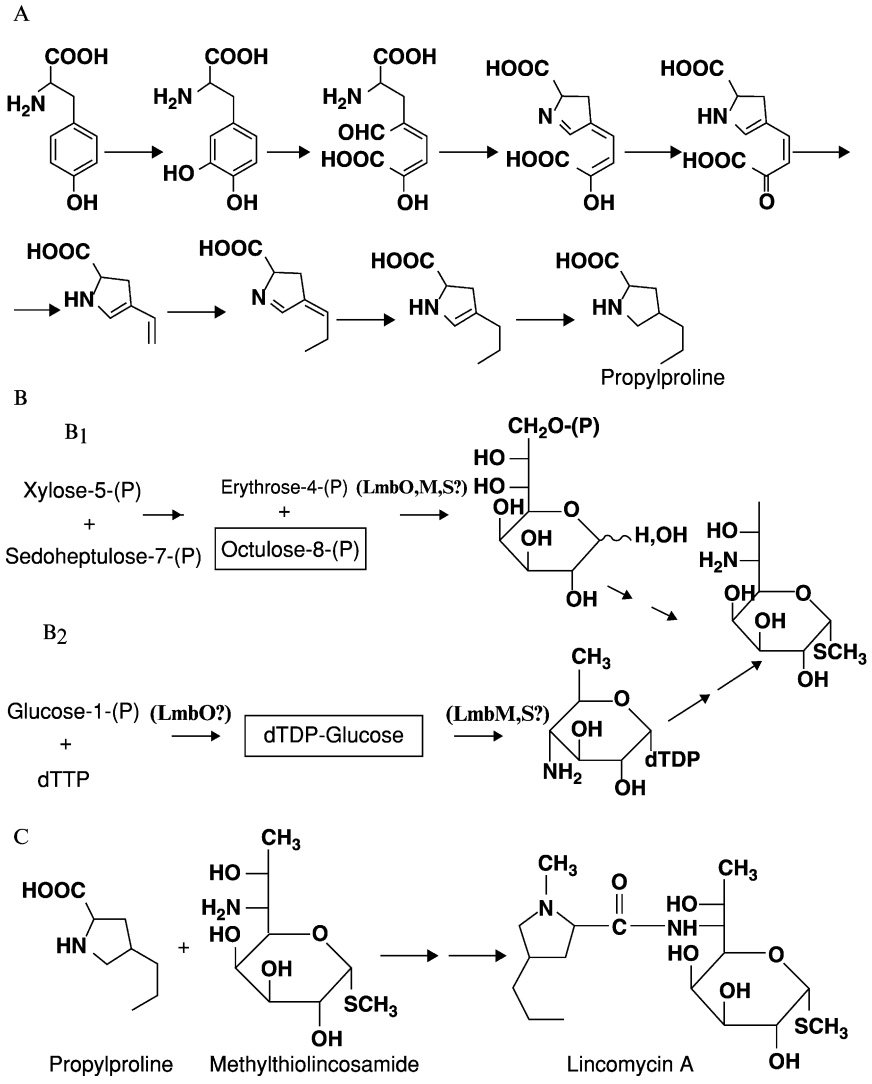


FIG. 1. Hypothetical biosynthetic pathway for lincomycin A. (A) Amino acid branch. (B) Sugar branch. Two alternative routes are proposed for methylthiolincosamide biosynthesis. (B_1) From intermediates of the pentose phosphate cycle *via* formation of a C_8 sugar precursor. (B_2) From dTDP-glucose *via* a 6-deoxyhexose pathway and a later extension of the carbon chain. The possible involvement of three of the gene products of the putative methylthio-lincosamide biosynthetic genes is indicated. (C) Condensation and final methylation.

A likely pathway leading from glucose to methylthiolincosamide was proposed by Brahme *et al.* (1984b), who studied the metabolic origin of methythiolincosamide by analyzing ^{13}C-^{13}C spin coupling patterns in methythiolincosamide and lincomycin-derived from biosynthetic experiments with D-($^{13}C_6$)glucose and the specific ^{13}C enrichments in methylthiolincosamide synthesized from specifically labeled substrates. The data obtained by means of both these approaches indicated that the C8 carbon skeleton of methythiolincosamide arises from condensation of a pentose unit (C5) and a C3 unit. The pentose unit could either be derived from glucose through the hexose monophosphate shunt as an intact unit or result from condensation of glyceraldehyde-3-phosphate with a C2 donor such as sedoheptulose-7-phosphate via transketolase reaction. The C3 unit probably arises from a suitable donor molecule such as sedoheptulose-7-phosphate and is added via a transaldolase reaction. According to the C3 unit donor origin, the unit could consist either of an intact C3 unit or a C2 unit combined with a C1 unit. The C3 and C5 units are then condensed, giving rise to octose, which is then converted to methylthiolincosamide. The final conversion of the C8-skeleton to methylthiolincosamide was predicted to involve isomerization of the octulose to octose, dephosphorylation and reduction of the C8 carbon, transamination of the precursor 6-ketooctose, and final thiomethylation of the C1 carbon. First attempts to find out the metabolic origin of the sugar moiety were by means of a combination of radioactive labeling and mass spectroscopy. It was determined that the S-methyl group of the methylthiolincosamide subunit is derived from C1 fragments at the oxidation state of methyl groups.

Sequencing of the lincomycin gene cluster, and amino acid sequence analysis of the putative protein products, led Peschke *et al.* (1995) to modify the aforementioned design of the biosynthetic pathway. Eight genes, *lmbL* through *lmbQ*, form a subcluster, which probably codes for a set of enzymes involved in sugar metabolism (Fig. 1). Putative proteins LmbO, LmbM, and LmbS show similarity to enzymes involved in the central steps of many NDP-6-deoxyhexose pathways, including sugar-activating pyrophosphorylases (LmbO), NDP-hexose dehydratases (LmbM), and (NDP-) ketosugar (or cyclitol) aminotransferases/dehydratases (LmbS). The corresponding genes are found mostly in clusters in both Gram-positive and Gram-negative bacteria, and their protein products are involved in the formation of other actinomycete secondary metabolites (Piepersberg, 1994; Pissowotzki *et al.*, 1991; Retzlaff *et al.*, 1993; Stockmann and Piepersberg, 1992) or of extracellular polysaccharides (Thorson *et al.*, 1993).

The genetic record led Peschke *et al.* (1995) to the conclusion that methylthiolincosamide biosynthesis involves a nucleotide (probably dTDP) activation step and a series of modification steps on dNTP-activated sugar intermediates. Thus, two totally different pathways, starting from either D-glucose or NDP-activation and modification of a C8 sugar intermediate, both represent routes leading to the production of methylthiolincosamide. The authors assume that the ^{13}C-labelling pattern in methylthiolincosamide published by Brahme *et al.* (1984b), suggesting that an octulose-phosphate intermediate is formed first could support glucose as a starter unit if there is rapid and efficient equilibrium in the hexose phosphate pool mediated by a hexose monophosphate shunt during the production phase. This type of rearrangement of labelling has been described for other streptomycete products directly derived from glucose (Rinehart *et al.*, 1992). The detection of a gene (lmbR) encoding a transaldolase-like enzyme in the ''sugar subcluster'' could support an alternative route via a pyranosidic octose intermediate that is NDP-activated and further modified in this form. Finally, a thiomethyl unit at the C1 position of the postulated (NDP-)6-amino-6,8-deoxyoctose intermediate would be added, which could be transferred from 5′-thiomethyladenosine, a side product of polyamine biosynthesis from S-adenosyl-methionine (Piepersberg and Distler, 1997).

C. Condensation and Final Modification

Formation of the amide bond between the carboxyl group of the aglycone propylproline and the amino group of the methylthiolincosamide yielding N-demethyllincomycin is apparently the penultimate step in lincomycin biosynthesis. N-demethyllincomycin-synthetase, catalyzing this reaction, is a complex of readily dissociable subunits with nonidentical molecular weights (Hausknecht and Wolf, 1986).

The final step in the pathway is N-methylation of N-demethyllincomycin, catalyzed by *S*-adenosylmethionine: N-demethyllincomycin methyl transferase (Patt and Horvath, 1985).

IV. Genetic Control of Lincomycin Biosynthesis

Similarly to other antibiotics (Martin and Liras, 1989) produced by actinomycetes, genes coding for lincomycin biosynthesis are clustered together in a single genomic region and closely linked to the corresponding resistance determinants (Chung and Crose, 1990; Wovcha *et al.*, 1986; Zhang *et al.*, 1992).

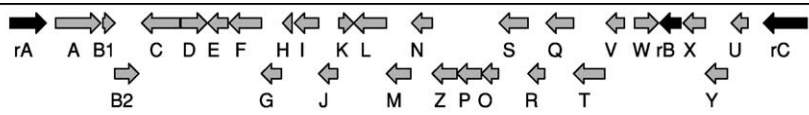


FIG. 2. The lincomycin gene cluster. Grey arrows designated A-Z indicate putative biosynthetic genes and black arrows designated rA-rC indicate genes coding for functions that impart lincomycin resistance phenotype.

The 35-kpb-long chromosomal region bearing a lincomycin-production gene cluster from the overproducing industrial strain *Streptomyces lincolnensis* 78–11 was cloned and sequenced by Peschke *et al.* (1995). The cluster contains 27 open reading frames with putative biosynthetic or regulatory functions (*lmb* genes) and three resistance (*lmr*) genes (Fig. 2). The genes designated *lmr*A and *lmr*C flank the cluster and appear to code for proteins probably involved in lincomycin export (Peschke *et al.*, 1995; Zhang *et al.*, 1992). The *lmr*B gene codes for a protein very similar to several 23S RNA methyltransferases (Zhang *et al.*, 1992). Four other lincomycin producers probably share the overall cluster organization; however, the clusters are embedded in nonhomologous chromosomal surroundings. DNA sequence analysis suggests a complicated transcription pattern with at least 12 possible transcription units.

On the basis of experiments using transposon 4560-mediated mutagenesis of the lincomycin production cluster of the strain derived from *Streptomyces lincolnensis* UC 5124, it is assumed that genes coding for enzymes involved in propylproline synthesis are located close to *lmr*A gene, whereas those controlling methylthiolincosamide synthesis are located further from it (Chung and Crose, 1990). Thus, biosynthetic genes seem to be organized according to functional pathways within the cluster region; however, the same experiments suggest that genes coding for subunits of the NDL synthetase, the key enzyme catalyzing propylproline and methylthiolincosamide condensation, are located at three widely separated loci (Chung *et al.*, 1997). The functions coded for by only a few of the aforementioned open reading frames were demonstrated experimentally. In addition to resistance genes, only the function of the genes *lmbB1* and *lmbB2*, coding for enzymes converting L-tyrosine and L-dihydroxyphenylalanine, was clarified (Neusser *et al.*, 1998). Gene *lmbJ* apparently codes for a specific N-demethyllincomycin methyltransferase (Janata *et al.*, 1999). Functions of other proteins coded for by lincomycin cluster genes can only be deduced.

V. Mechanism of Action

Lincosamides belong to antibiotics that block microbial protein synthesis. Protein synthesis includes a large number of steps from activation of amino acid monomers by aminocayl-tRNA synthetases to many steps of chain initiation, elongation, and chain termination of the grown polypeptides on the ribosome. Antibiotics interrupt the timing and specificity of any of these steps, and such disruptions decelerate the growth or are lethal to the microorganism. A molecular mechanism by which clindamycin inhibits ribosomal protein biosynthesis in prokaryotic microorganisms has therefore been unclear. However, recently it was shown that clindamycin's three-dimensional structure closely resembles the L-Pro-Met and the D-ribosyl ring of adenosine (Fitzhugh, 1998) biomolecules, which occur proximate to 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. This finding strongly suggests that clindamycin and other lincosamides act as structural analogs of the 3′-ends of L-Pro-Met-tRNA and deacylated-tRNA as they are positioned during an initial phase of pretranslocation in the peptide elongation cycle. Clindamycin thus constitutes a structural analog of an intermediate state in protein biosynthesis. 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 release of peptidyl-tRNA intermediates prematurely by blocking the approach to the elongating peptide's exit tunnel (Schlunzen *et al.*, 2001). Erythromycin also blocks assembly of 50S subunits, probably through its interaction with 23S rRNA. 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. A point mutation at A_{2058} of 23S rRNA induces a triple resistance to macrolides, lincosamides, and streptogramin B. Schlunzen *et al.* (2001) provided a direct proof with the cocrystal structure of the lincosamide antibiotic clindamycin, in which the 2′- and 3′-hydroxyl groups of the antibiotic sugar moiety form hydrogen bonds with the same exocyclic N_6 amino group of A_{2058}. Clindamycin and erythromycin binding show a partial physical overlap. Clindamycin has separately been known to interact with both the A site and the P site of the peptidyltransferase center. In accordance with their mechanism of action, bacteria quite often

develop cross-resistance to macrolides, lincosamides, and streptogramin B. Lincomycin and clindamycin share a common mechanism in sensitive microorganisms and also exhibit a similar antibacterial spectrum. Nevertheless, slight differences in their antimicrobial activity exist as clindamycin also affects some protozoa—for example, *Toxoplasma gondii, Plasmodium falciparum*, and *Pneumocystis carinii*. Administration of 450–600 mg of clindamycin every 6 h and 15–30 mg of primaquine base applied once a day were used successfully for the treatment of infections caused by *Pneumocystis carinii* (Fishman, 1998), whereas lincomycin did not exhibit any activity.

Remington (1990) demonstrated that a combination of clindamycin with primaquine is highly effective in the treatment of pneumonia caused by *Pneumocystis carinii*, even in AIDS patients.

Clindamycin inhibits bacterial protein synthesis and acts specifically on the 50S subunit of the bacterial ribosome, most likely by affecting the process of peptide chain initiation. It may also stimulate dissociation of peptidyl-tRNA from ribosomes (Menninger and Coleman, 1993). *Escherichia coli* exposed to subminimal inhibitory concentrations of clindamycin shows a decreased adherence to buccal mucosal cells, which may be due to protein synthesis inhibition.

Presumably it was suppression of protein synthesis that also enabled Sanders *et al.* (1983) to show that clindamycin is an effective *in vitro* inhibitor of the derepression of bacterial β-lactamases that would otherwise have been produced by certain nonfastidious Gram-negative bacilli exposed to various β-lactam antibiotics. Similarly, Schlievert and Kelly (1984) showed inhibition of toxin production in toxic shock syndrome-producing strains of *Staphylococcus aureus* by concentrations of clindamycin that do not inhibit bacterial growth.

Macrolides and lincosamides are first-choice bacteriostatic antibiotics used in veterinary dermatology. The main antibiotics of these classes are erythromycin, lincomycin, clindamycin, and tylosin. They are well absorbed if administered orally and are able to penetrate well into infected skin. Their spectrum of action comprises bacteria commonly associated with skin infections, including staphylococci. Their main disadvantage is a rapid development of bacterial resistance and occasional gastrointestinal upset (Noli and Boothe, 1999).

Macrolides, fluoroquinolones, rifamycins, tetracyclines, trimethoprim-sulfamethoxazole, and clindamycin were described as antimicrobial agents of preference for the dermatologist (Epstein *et al.*, 1997).

Clindamycin administration results in changes in intestinal microflora. Numbers of enterococcal species increase and those of all anaerobes decrease (Nord and Heimdahl, 1986).

Some antibiotics also exhibit immunomodulatory effects, clindamycin among them (VanVlem *et al.*, 1996).

VI. Resistance Against Lincosamides

In general, basic mechanisms of antibiotic resistance include microbial cell impermeability, target site modification, and enzymatic modification or destruction of the antibiotic and its increased efflux.

The main type of resistance to lincomycin and clindamycin is the so-called MLS_b resistance that renders sensitive microorganisms resistant to macrolides, lincosamides, and streptogramin B (hence MLS_b resistance). It is monomethylation or dimethylation of the N_6 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*). Increased efflux of lincosamides was detected in some microorganisms resistant to them. The occurrence of some genes involved in MLS_B resistance in methicillin-resistant strains of *Staphylococcoccus aureus* was investigated, and it was shown that, in the Czech Republic, genes *ermC* and *ermA* are the most frequent determinants of MLS resistance (up to 90% cases) and that gene *msrA*, encoding the protein responsible for the active excretion by the resistant cells of macrolides and streptogramins but not of lincosamides, is less common. Gene *linA*, whose protein product modifies and thus inactivates lincosamide antibiotics only, is an additional resistance gene that is less frequent (Novotna *et al.*, 2002).

Clindamycin and lincomycin share a common or overlapping binding site on the ribosome. As already mentioned, a ribosomal mutation makes the ribosome insensitive to clindamycin. Expression of clindamycin-resistance in Gram-positive cocci may be constitutive or inducible. Staphylococci can also owe their resistance to clindamycin because of enzymatic inactivation of the drug, the enzyme production being specified by small nonconjugative plasmids. The third resistance mechanism to clindamycin involves active efflux of the antibiotic from the periplasmic space. This mainly occurs in Gram-negative bacteria (Leclercq and Courvalin, 1991).

Lincomycin resistance in clinical isolates of staphylococci and streptococci has been recognized for several decades. This resistance is plasmid mediated and is encoded on transposons. The resistance

results from the induction of an enzyme that is normally repressed. The methylated RNA binds lincomycin-type drugs less well than does the nonmethylated RNA. The induction of resistance varies by species, and in most Gram-positive species, erythromycin is a more-effective inducer of resistance than clindamycin. The plasmids that mediate lincomycin resistance in streptococci and staphylococci are highly similar structurally, indicating that they could have been readily transferred among strains of these species.

Inactivation (resistance) of lincosamides by the products of the *linA* (encoding 3-lincomycin 4-clindamycin O-nucleotidyltransferase) genes of *Staphylococcus aureus* is one of the resistance mechanism in this bacterium (Matsuoka, 2000).

VII. Biological Activity and Applications

When usual doses are applied, both lincomycin and clindamycin exhibit bacteriostatic activity (Table III). At higher concentrations that can still be reached *in vivo*, their effect may be even bactericidal. However, the onset of the bactericidal effect is delayed and is less complete than that of, for example, β-lactams. It increases generally in parallel with an 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 at a substantially wider concentration range of clinical therapeutical doses.

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Macrolides, fluoroquinolones, rifamycins, tetracyclines, trimethoprim-sulfamethoxazole, and clindamycin have been described as antimicrobial agents of preference for the dermatologist (Epstein *et al.*, 1997).

Clindamycin administration results in changes in intestinal microflora. Numbers of enterococcal species increase and those of all anaerobes decrease (Nord and Heimdahl, 1986).

Lincomycin was also used as an inhibitor of protein synthesis in experiments concerning photoinactivation in plants and algae (Kato *et al.*, 2002; Sicora *et al.*, 2003).

TABLE III

EFFECT OF LINCOMYCIN AND CLINDAMYCIN ON SOME COMMON PATHOGENIC BACTERIA

	MIC (μg/ml)	
Test organism	Lincomycin	Clindamycin
Gram-positive	Generally sensitive	Generally sensitive
Bacillus anthracis	0.25–8.0	0.25–5.0
Staphylococcus aureus	0.2–3.2	0.04–1.6
Staphylococcus epidermidis	0.4–1.8	0.1–0.2
Streptococcus agalactiae	0.1–0.2	0.02–0.1
Streptococcus pneumoniae	0.01–0.8	0.002–0.1
Streptococcus pyogenes	0.04–0.8	0.01–0.2
Streptococcus viridans	0.02–1.0	0.005–0.2
Gram-negative	Generally resistant	Generally resistant
Escherichia coli	1000	64
Haemophilus influenzae	4–16	0.5–16.0
Klebsiella pneumoniae	8	125
Neisseria gonorhoeae	8–64	0.5–4.0
Neisseria meningitis	>32	4
Proteus vulgaris	1000	250
Pseudomonas aeruginosa	>1000	1000
Salmonella schottmuelleri	125	64

The values in Table III can serve for a general orientation only. They were obtained from a number of experimental papers in which different strains were used and the data often varied substantially.

VIII. Gram-Positive Bacteria

Clindamycin is active against most of the following bacteria: *Staphylococcus aureus, Streptococcus pyogenes, S. pneumoniae, S. viridans* and *S. bovis, Corynebacterium diphtheriae, Enterococcus durans, Bacillus anthracis, B. cereus*, and the *Nocardia* spp., but unfortunately, it is inactive against *Enterococcus faecalis* and *E. faecium* (Gigantelli *et al.*, 1991). In stomatology, clindamycin can be used for the treatment of infections caused by *Bacillus melaninogenicus* and *B. fragilis*.

Bacterial skin and skin structure infections are caused by aerobic staphylococci and streptococci (*Streptococcus pyogenes* and *Staphylococcus aureus*), with aerobic Gram-negative bacilli and anaerobes being involved in more complicated infections. Systemic therapy with lincosamides (clindamycin) has been the cornerstone of the treatment of these infections for many years (Guay, 2003).

Staphylococcus aureus and *Streptococcus pyogenes* cause a number of serious infections, such as necrotizing fascitis and toxic shock syndrome, which are associated with the release of bacterial toxins. Animal studies showed that clindamycin is more effective in treating these severe infections than are other drugs (Coyle, 2003).

Bacillus anthracis infection can occur in three forms: cutaneous, gastrointestinal, and inhalational, depending on the mode of infection. Anthrax is a zoonotic disease but, unfortunately, the inhalation form can also be used as a biological warfare agent. In addition to other antibiotics, clindamycin was used for treatment (Brook, 2002a).

Severe infections caused by *Streptococcus pyogenes* (group A streptococci) can be treated with clindamycin, acting as an inhibitor of the synthesis of protein M and of extracellular proteins (Bouvet, 1996).

Pneumonia caused by *Bacillus cereus* could be treated with clindamycin (Bastian *et al.*, 1997).

Although tests of clindamycin resistance in streptococci are not generally performed in clinical practice, resistance to clindamycin has already been detected in strains of the following species: *Streptococcus pyogenes*, strains of group B streptococci, and strains of *S. pneumoniae*. Pneumococci that are multiply resistant to many antibiotics, including clindamycin, were reported from South Africa, but in most areas in the United States they are still clindamycin-sensitive. Staphylococci resistant to clindamycin are more common, and clindamycin sensitivity tests are always performed (Reeves *et al.*, 1991).

Type 3 pneumococci produce a capsule composed of cellobiuronic acid units connected in a β (1 $\rightarrow$ 3) linkage. The genes implicated in the biosynthesis of the type 3 capsule (cap3 genes) were cloned, expressed, and biochemically characterized. The three cap3 genes designated as cap3ABC form an operon. The Cap3A, Cap3B, and Cap3C proteins were biochemically characterized as a UDP-glucose dehydrogenase, the type 3 polysaccharide synthase, and a glucose-1-P uridyltransferase, respectively. The Cap3B protein was expressed in *E. coli*, and pneumococcal type 3 polysaccharide was synthesized in this heterologous system. When a recombinant plasmid containing cap3B (pLSE3B) was introduced into encapsulated pneumococci of types 1, 2, 5, or 8, the lincomycin-resistant transformants showed a type 3 capsule in addition to that of the recipient type. Unencapsulated (S2) laboratory strains of *S. pneumoniae* also synthesized a type 3 capsule when transformed with the pLSE3B plasmid (Garcia *et al.*, 1997).

The increasing prevalence of clindamycin-resistant bacteria (resistant *Streptococcus pneumoniae* in particular) is leading to new approaches to the management of common respiratory infections in the outpatient setting (Green and Wald, 1996).

Clindamycin is an effective therapy for community-acquired, methicillin-resistant *Staphylococcus aureus*, but there is a risk of development of clindamycin resistance during the treatment of these bacteria (Marcinak and Frank, 2003).

Emerging treatments for streptococcal toxic shock syndrome (caused by, for example, *Streptococcus pyogenes*) include administration of clindamycin and intravenous γ-globulin (Stevens, 2000).

Streptococcus pyogenes, particularly the capsule and protein M, as well as streptococcal toxins, cause severe septic and toxic syndromes. Clindamycin should be used in case of septic shock (Veyssier-Belot *et al.*, 1999).

The efficacy of clindamycin and the failure of penicillin to treat a severe group A streptococcal infection and streptococcal toxic shock syndrome were described (Stevens, 1996).

An enhanced bactericidal response against β-hemolytic streptococci has been found with a combination of penicillin and clindamycin (Seal, 2001).

Resistance of enterococci and staphylococci to many antibiotics including clindamycin was described by McManus (1997).

Antibiotic resistance of different strains of *Bacteroides, Prevotella*, and *Porphyromonas* species and *in vitro* antimicrobial susceptibility to many antibiotics, including clindamycin, were reviewed by Falagas and Siakavellas (2000).

The true fungi and acid-fast and poorly Gram-stainable *Mycobacterium tuberculosis* are clindamycin-resistant, but clindamycin has some activity against *M. leprae*.

IX. Gram-Negative Bacteria

In general, aerobic Gram-negative bacteria are resistant to clindamycin. It was described that *in vitro* clindamycin is more active against *H. influenzae* than lincomycin. *Campylobacter jejuni* is sensitive to clindamycin, but *E. coli* is much more resistant (128 μg/ml) compared with *C. jejuni* (8 μg/ml). *Capnocytophaga canimorosus* causing bacteremic illness and dog sickness or other animal diseases is clindamycin-sensitive. Flavobacteria may also be clindamycin-sensitive (Sheridan *et al.*, 1993).

Clindamycin has good activity against the *Bacteroides fragilis* group of anaerobic bacteria (2 μg/ml). Unfortunately, the number of clindamycin-resistant strains increases with time, but *B. fragilis* still remains one of the most sensitive bacteria as compared with *B. thetaiotaomicron, B. ovatus, B. vulgatus*, and *B. distasonis* (Tanaka-Bandoh *et al.*, 1995). For instance, in the United States, the resistance is very low, varying to up to 25% of strains. Unfortunately, as usually, it increases proportionally with time. Thus in the *B. fragilis group* it increased from 4% to 38% in 10 years. A similar situation has also been observed in other parts of the world (Patey *et al.*, 1994; Turgeon *et al.*, 1994).

Clindamycin is quite active against other Gram-negative anaerobes such as *Prevotella disiens* and *P. melaninogenica* and the *Fusobacterium* spp. *Bacteroides gracilis* may be clindamycin-sensitive, but some strains are resistant.

Additional Gram-negative bacteria comprising strains of *Butyrivibrio, Succinimonas*, and *Anaerovibrio* can be sensitive to clindamycin.

Necrotizing fascitis continues to occur due to β-hemolytic streptococci but is now also recognized as being due to *Vibrio* spp. in fishermen and those in contact with warm water in the Gulf of Mexico and Southeast Asia, including Hong Kong. The mechanism of *Bacteroides* resistance to clindamycin is usually as in Gram-positive bacteria (Jimenez-Diaz *et al.*, 1992; Reig *et al.*, 1992a). The resistance gene in *Bacteroides* spp. can be located on plasmids or on the chromosome; it can be transferred between species by a plasmid or transposon.

Leng *et al.* (1975) used combinations of clindamycin with gentamicin against *Enterobacteriaceae* and *Pseudomonas aeruginosa* and demonstrated synergism. Klastersky and Husson (1977) showed that gentamicin did not interfere with the activity of clindamycin against *B. fragilis*, and that clindamycin did not influence the activity of gentamicin against *E. coli*.

Adjunctive use of clindamycin, along with mechanical debridement is recommended for the treatment of *Actinobacillus actinomycetemcomitans* (Gram-negative, facultative anaerobic bacterium)-associated periodontitis as an acceptable therapeutic regimen (Walker and Karpinia, 2002).

Laboratory results could be used in clinical practice by comparing the MIC_{50} of the germs regularly encountered in bone infections (staphylococci, streptococci including enterococci, Gram-negative bacilli, *P. aeruginosa*, and *H. influenzae*) with concentrations obtained in the different studies. In these studies it was described that clindamycin has a moderate bone diffusion (Boselli and Allaouchiche, 1999). *Mycoplasma* and *Ureaplasma urealyticum* are clindamycin-resistant;

on the contrary, clindamycin has some activity against *Chlamydia trachomatis* (Rice *et al.*, 1995) or *Coxiella burnetii*.

X. Anaerobic Bacteria

Clostridium tetani and *C. perfringens* are sensitive to clindamycin, but some *C. perfringens* strains and strains of *C. sporogenes, C. tertium, C. bifermentans, C. novyi, C. ramosum*, and *C. sordelli* may be clindamycin-resistant.

A systematic review of studies that investigated the association of clindamycin-like antibiotics with hospital-acquired *Clostridium difficile* diarrhea was undertaken to summarize the strength of the evidence for this relationship (Thomas *et al.*, 2003).

Clostridium difficile is responsible for 300,000 to 3,000,000 cases of diarrhea and colitis in the United States every year. Antibiotics most frequently indicated for this infection are clindamycin, ampicillin, amoxicillin, and cephalosporins (Mylonakis *et al.*, 2001).

Clindamycin was an effective drug in the treatment of Gram-positive anaerobic infections (e.g., *Clostridium perfringens*). Very rapidly, the anti-anaerobic armamentarium was extended with clindamycin, cefoxitin, imipenem, and co-amoxyclavin or piperacillintazobactam. The resistance rate to metronidazole and imipenem remains low, but clindamycin has seen an important decrease in bacterial susceptibility (Bryskier, 2001).

Clostridium difficile is now established as a major nosocomial pathogen. *C. difficile* infection is seen almost exclusively as a complication of antibiotic therapy and is particularly associated with clindamycin and third-generation cephalosporins (Freeman and Wilcox, 1999).

Agents with a high potential to induce *Clostridium difficile*–associated disease include aminopenicillins, cephalosporins, and clindamycin (Job and Jacobs, 1997).

Other anaerobic Gram-positive organisms such as *Peptococcus, Peptostreptococcus, Eubacterium, Propionibacterium, Bifidobacterium* and *Lactobacillus* spp., *Actinomyces israelii* or *Bifidobacterium*, and *Eubacterium* spp. (Brook and Frazier, 1993) are usually sensitive to clindamycin. Naturally, even here, resistant strains have been described—for example, in *Peptostreptococcus* spp. (Reig *et al.*, 1992b) or *Lactobacillus* spp.

Vaginal bacterial infections are usually caused by mixed bacterial populations, including *Peptostreptococcus* sp., *Peptococcus* sp., *B. fragilis*, and of the aerobic bacteria by *Streptococcus viridans, S. agalactiae*, and less by *S. pyogenes* and other enterobacteria. The mixed

population can also contain enterococci and staphylococci. With the exception of enterococci, the population is to a considerable extent sensitive to a combination of clindamycin with an aminoglycosidic antibiotic. Clindamycin was also found to inhibit *Chlamydia trachomatis*. Bacterial vaginosis is an alteration of the vaginal flora, where the normally predominant lactobacilli are replaced by a mixture of organisms including *Gardnerella vaginalis* and anaerobes and is treated with metronidazole or clindamycin (Priestley and Kinghorn, 1996). In bacterial vaginosis the normal hydrogen peroxide-producing *Lactobacillus* sp. in the vagina is replaced with high concentrations of characteristic sets of aerobic and anaerobic bacteria. It also occurs in women treated, for example, by orally administered clindamycin (McGregor and French, 2000).

Increased doses of clindamycin and lincomycin (at least 8 g per day in adults) have to be administered for the treatment of *B. fragilis* infections, and even then the effect is not guaranteed.

In cases of anaerobic sepsis, usually caused by *B. fragilis* or *Peptostreptococcus* sp., the application of clindamycin as the first choice antibiotic is fully justified.

In many patients with acne, caused by resistant *Propionibacterium acnes*, continued treatment with antibiotics such as clindamycin can be inappropriate or ineffective (Cooper, 1998).

Clostridium difficile may be clindamycin-sensitive or -resistant, and the proportion of sensitive strains has varied from 10% to 90% in different studies. During outbreaks of diarrhea associated with *C. difficile*, the strains are usually clindamycin-resistant, and they contain a plasmid, probably located on the chromosome. This codes for transferable macrolide-lincosamide-streptogramin B (MLS_B) resistance. This resistance can be transferred from *C. difficile* to *Staphylococcus aureus*. In one study, almost all of 161 isolates of *C. difficile* of serogroups A, F, G, H, and X were susceptible to clindamycin and other antibiotics, but 32 toxigenic isolates of serogroup C were clindamycin resistant.

The microbiology, diagnosis, and management of bacteremia caused by anaerobic bacteria (*Bacteroides fragilis, Peptostreptococcus* sp., *Clostridium* sp., and *Fusobacterium* sp.) in children were reviewed by Brook (2002b).

XI. Protozoa and Other Organisms

Clindamycin has been used as an antimalarial drug (Lell and Kremsner, 2002). It was found effective in animals infected with chloroquine-resistant and chloroquine-sensitive *Plasmodium falcipar-*

um. It is also effective against *P. vivax* but not against the exo-erythrocytic parasites.

In *P. falciparum* clindamycin appears to be quite effective in the treatment of semi-immune subjects, and it enhances quinine activity (Patenotte *et al.*, 1995).

Malaria caused by chloroquine-resistant strains can be treated successfully with combinations of clindamycin with a ''classical'' antimalarial drug (Mordmuller *et al.*, 1998).

Clindamycin is effective in experimental toxoplasmosis in mice. In cultured mammalian cells, clindamycin reduces the level of replication of *Toxoplasma gondii* affecting protein synthesis of free parasites and also impairs the ability of the parasite to infect host cells. Infections caused by *Toxoplasma gondii* can be treated with clindamycin (Fung and Kirschenbaum, 1996). *T. gondii* clindamycin-resistant mutants can be selected that usually exhibit cross-resistance to spiromycin and azithromycin (Fichera *et al.*, 1995).

The influence of antimicrobial agents on replication and stage conversion of *Toxoplasma gondii* was described by Gross and Pohl (1996). Molecular genetic tools for the identification and analysis of drug targets in *Toxoplasma gondii* were reviewed by Roos (1996).

Human babesiosis is an important emerging tick-borne disease. *Babesia divergens*, a parasite of cattle, has been implicated as the most common agent of human babesiosis in Europe and/or *Babesia microti* in the United States, causing severe disease in splenectomized individuals. Human babesiosis can be treated by clindamycin administered intravenously (Uguen *et al.*, 1997). Current treatment for babesiosis is focused on a regimen of clindamycin and quinine (Kjemtrup and Conrad, 2000).

XII. Conclusion and Future Prospects

Lincomycin and clindamycin are clinically important antibiotics. According to its worldwide production, the semisynthetic lincosamide derivative clindamycin is one of the 20 most important antibiotic compounds. They are active against most Gram-positive bacteria and against the genera *Staphylococcus* and *Streptococcus* in particular. They do not affect Gram-negative bacteria but exhibit a significant antibiotic activity against some anaerobic bacteria. They are used therapeutically, especially in cases where synergistic effects of a mixed anaerobic and aerobic microflora are anticipated (prevention of intraabdominal infection after surgery, stomatological infections, anaerobic sepsis, skin and mucosa infections, and especially infections of bone

and articular infections). Lincomycin and clindamycin are also useful alternatives to penicillin and its derivatives in the treatment of upper respiratory tract infections in patients with allergy to penicillin. As compared with lincomycin, clindamycin is highly effective in the treatment of toxoplasmosis and pneumocystosis in AIDS patients. Clindamycin and some of its derivatives seem to be promising for the treatment of malaria caused by *Plasmodium falciparum*, even of strains that developed resistance to chloroquine, sulfonamides, and pyrimethamine. By means of chemical modification of lincomycin, a number of derivatives with improved properties were obtained, whereas biotransformation has so far been less successful. However, with the current knowledge of gene clusters specifying biosynthesis of lincomycin and the related antibiotic celesticetin, one can easily imagine production of new derivatives of lincosamides by genetic engineering, namely by combination of parts of the lincomycin cluster with those of the celesticetin cluster, resulting in production of new derivatives of lincosamides. The knowledge of genomes of more than 100 pathogenic bacteria will make it possible to direct the search for new antibiotics specific for new targets in the pathogens. In addition, the recent completion of the genomes of *Streptomyces coelicolor* and *Streptomyces avermitilis* revealed that new secondary metabolites that had not yet been described are coded for by specific genes. Thus, not only hybrid antibiotics but also completely new compounds can still be discovered in the near future. In this respect it appears to the authors that lincosamides are not only useful antibiotics, namely 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.

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