SHORT ARTICLES

BACTERIOLOGICAL ASSESSMENT OF CLINDAMYCIN, A NEW LINCOMYCIN DERIVATIVE

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CLINDAMYCIN, or 7(S)-chloro-7-deoxylincomycin is a recently isolated derivative of lincomycin, with the empirical formula $C_{18}H_{33}O_5N_2ClS \cdot HCl \cdot H_2O$. It differs from lincomycin in the presence of a chlorine atom in place of a hydroxyl group at position 7. The compound has the trade name Dalacin-C. It is claimed to be more active than lincomycin against a range of Gram-positive bacteria. Accordingly, it may be used in lower dosage and is less likely to produce the intestinal irritation that may accompany lincomycin therapy (Duncan and Jeans, 1965).

MATERIALS AND METHODS

Antibiotics. Clindamycin was obtained in powder form from the Upjohn Company, Kalamazoo, Michigan; the powder contained 880 μ g clindamycin per mg. Lincomycin with a potency of 888 μ g per mg was obtained from the Upjohn Company, Kalamazoo, Michigan. Erythromycin powder with a potency of 920 μ g per mg was obtained from Abbott Laboratories Ltd, Montreal. Benzylpenicillin powder with a potency of 1000 μ g per mg was obtained from Glaxo Laboratories, England. Oxacillin powder with a potency of 870 μ g per mg was obtained from Bristol Laboratories, Syracuse, N.Y.

Antibiotic plates. The antibiotics were dissolved in sterile distilled water to make stock solutions of appropriate strength. Samples of these stock solutions were added to flasks of melted and cooled (50°C) tryptose agar to give concentrations as follows: clindamycin—0.025, 0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 μ g per ml; lincomycin—0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 μ g per ml; erythromycin—0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 μ g per ml; benzylpenicillin—0.125, 0.25, 0.5, 1, 2, 4, 8 and 16 μ g per ml; oxacillin—1 μ g per ml.

The antibiotic media were poured in square plastic plates (Falcon Plastics Ltd) marked with a 36-square grid. Plates were stored at 4°C and used within 2 wk of preparation. They were dried at 37°C for 2 hr immediately before use. Antibiotic plates to be inoculated with *Haemophilus influenzae* strains included 5 per cent. by volume of Fildes' extract in the basal medium.

Bacteria. The bacteria tested were all isolated from specimens received in the Diagnostic Microbiology Laboratory, Kingston General Hospital. All isolates of any one species were from different patients.

Inoculation of plates. Each isolate was streaked on a tryptose agar plate for purification, and six or seven representative colonies were transferred from this plate to a tube of tryptose broth. This broth was incubated for 16–18 hr at 37°C. The overnight broth culture was diluted by ten-fold steps, in quarter-strength Ringer solution, to 10^{-4} . By means of a multiple inoculator, the undiluted broth culture of each isolate and its four serial ten-fold dilutions were inoculated on each antibiotic plate and on a control tryptose agar plate containing no drugs. Dilution sets of six strains were inoculated on to each plate. *H. influenzae* strains were purified and subcultured on media containing 5 per cent. by volume of Fildes' extract. All plates were incubated at 37°C.

Reading of plates. After 24 hours' incubation, plates were read by naked-eye examination.

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The presence and degree of growth on the antibiotic plates were recorded in terms of growth on the control plate.

Minimum inhibitory concentrations (MIC) were defined in two ways: (1) as the smallest tested concentration causing complete inhibition of growth (*CI* MIC); (2) as the smallest tested concentration causing reduction of growth (*RG* MIC). MICs of each antibiotic were recorded in these ways for all inocula of all the bacterial isolates tested.

RESULTS

Response of coagulase-positive staphylococci to clindamycin

The results of MIC determinations for clindamycin against 300 strains of coagulase-positive staphylococci are presented in table I, which shows that clindamycin was

TABLE I
Minimum inhibitory concentrations (MICs) of clindamycin for 300 strains of coagulase-positive staphylococci

Degree of inhibition of growth for which MIC determined	Bacterial inoculum*	Number of strains showing stated degree of inhibition of growth at a clindamycin concentration (μg per ml) of							
		0.025	0.05	0.1	0.2	0.4	0.8	1.6	>1.6
Complete (CI)	10^0	0	6	187	87	8	1	2	9
	10^{-1}	4	31	245	13	2	0	2	3
	10^{-2}	7	89	192	6	1	2	0	3
	10^{-3}	12	138	139	7	1	0	0	3
	10^{-4}	28	185	80	4	0	0	1	2
Reduction (RG)	10^0	5	105	172	12	2	1	1	2
	10^{-1}	24	181	88	4	1	0	0	2
	10^{-2}	75	204	18	1	0	0	0	2
	10^{-3}	190	106	2	0	0	0	0	2
	10^{-4}	289	9	0	0	0	0	0	2

* Dilution of overnight broth culture.

highly active against nearly all the 300 strains. There was a gradual fall of MIC as the inoculum decreased and the *RG* MIC was in general lower than the *CI* MIC. With the heaviest inoculum, only nine strains were resistant to a drug concentration above 1.6 μg per ml. Of these, seven showed some reduction in growth at this concentration, but the other two strains were completely resistant to 100 μg per ml.

Table II shows the number of strains sensitive and resistant to concentrations of the antibiotics that may be expected in the bloodstream after normal dosage. It was anticipated here that clindamycin would be used in one-quarter the dosage of lincomycin.

Median MICs of four antibiotics for coagulase-positive and coagulase-negative staphylococci and for Streptococcus faecalis

Table III shows the median MICs of all four drugs for the coagulase-positive staphylococci, the coagulase-negative staphylococci and *Strep. faecalis* isolates. The median MIC of clindamycin for the staphylococci, irrespective of coagulase reaction, was 4–16 times lower than the median MIC of lincomycin, depending on inoculum and criterion of inhibition. For any one drug acting on any of these three species, the ratio of median MICs for the largest and smallest inocula was rarely higher than 4. The notable exception occurred, not unexpectedly, with benzylpenicillin and coagulase-positive staphylococci, where the median

TABLE II

Comparative sensitivity of 300 strains of coagulase-positive staphylococci to usual plasma concentrations of clindamycin, lincomycin, erythromycin and benzylpenicillin, using undiluted broth culture as inoculum

Antibiotic		Number of strains that were, to judge from			
Name	Concentration (μg per ml)	complete inhibition tests,		reduced growth tests,	
		sensitive	resistant	sensitive	resistant
Clindamycin	0.4	288	12	296	4
Lincomycin	1.6	253	47	280	20
Erythromycin	0.8	238	62	261	39
Penicillin	0.5	92	208	98	202

TABLE III

Median minimum inhibitory concentrations of clindamycin, lincomycin, erythromycin and benzylpenicillin for 300 strains of coagulase-positive staphylococci, 50 strains of coagulase-negative staphylococci and 50 strains of Streptococcus faecalis

Organism	Inoculum*	Median minimum inhibitory concentration (μg per ml), judged by complete inhibition of growth (CI) or reduced growth (RG), of							
		clindamycin		lincomycin		erythromycin		benzylpenicillin	
		CI	RG	CI	RG	CI	RG	CI	RG
Coagulase-positive staphylococci	10^0	0.1	0.1	1.6	0.8	0.4	0.4	16	8
	10^{-1}	0.1	0.05	0.8	0.8	0.4	0.2	2	0.5
	10^{-2}	0.1	0.05	0.8	0.4	0.4	0.2	0.5	0.25
	10^{-3}	0.075	0.025	0.8	0.2	0.2	0.1	0.25	0.125
	10^{-4}	0.05	0.025	0.8	0.1	0.2	0.1	0.125	0.125
Coagulase-negative staphylococci	10^0	0.1	0.1	0.8	0.4	0.4	0.2	2	0.5
	10^{-1}	0.1	0.05	0.8	0.4	0.4	0.2	0.25	0.125
	10^{-2}	0.1	0.025	0.4	0.2	0.2	0.1	0.125	0.125
	10^{-3}	0.05	0.025	0.4	0.15	0.2	0.1	0.125	0.125
	10^{-4}	0.05	0.025	0.4	0.1	0.2	0.1	0.125	0.125
<i>Streptococcus faecalis</i>	10^0	1.6	1.6	>6.4	>6.4	0.8	0.4	2	1
	10^{-1}	1.6	1.6	>6.4	>6.4	0.4	0.3	2	1
	10^{-2}	1.6	0.8	>6.4	6.4	0.4	0.2	2	0.5
	10^{-3}	1.6	0.4	>6.4	6.4	0.4	0.2	2	0.5
	10^{-4}	1.6	0.4	>6.4	3.2	0.4	0.1	2	0.5

* Dilution of overnight broth cultures.

MIC ratios were 128 and 64 for the *CI* and *RG* criteria respectively. The action of benzylpenicillin on *Strep. faecalis* was very little influenced by inoculum size.

Most of the median MIC values for staphylococci were below the antibiotic concentrations readily attainable in the human bloodstream after normal dosage. Clindamycin and lincomycin were relatively inactive against *Strep. faecalis*, but all strains were inhibited by erythromycin, irrespective of inoculum.

Response of staphylococci and Strep. faecalis to oxacillin

Of the 300 strains of coagulase-positive staphylococci 298 were sensitive to oxacillin 1 μg per ml. The remaining two were moderately resistant, i.e., showed a fine haze of growth on oxacillin agar after overnight incubation. Forty-six of the 50 coagulase-negative staphylococci were sensitive to oxacillin 1 μg per ml, two strains were moderately resistant and two completely resistant. Forty-six of the *Strep. faecalis* strains were fully resistant to oxacillin 1 μg per ml, the other four showing moderate resistance.

Response of various bacterial species to clindamycin

Of 19 strains of *Streptococcus pneumoniae* tested against clindamycin, all were sensitive to 0.025 μg per ml at inocula ranging from 10^{-1} to 10^{-4} of an overnight broth culture; the undiluted cultures of 17 strains were also inhibited by 0.025 μg per ml; the remaining two required 0.05 μg per ml for complete inhibition. On the reduced growth criterion, all 19 strains were inhibited by 0.025 μg per ml, at all inocula tested.

Of 21 strains of β -haemolytic streptococci, 20 strains were completely inhibited by 0.025 μg per ml of clindamycin at all inocula tested; one strain required 0.05 μg per ml for complete inhibition of the undiluted inoculum, whereas all diluted inocula were inhibited by 0.025 μg per ml. On the reduced growth criterion, all 21 strains were inhibited by 0.025 μg per ml at all inocula tested.

When tested against 25 strains of viridans streptococci, clindamycin at 0.025 μg per ml completely inhibited 21 strains in undiluted cultures, 24 strains at 10^{-1} and 10^{-2} inocula, and all 25 strains at higher dilutions. A concentration of 0.05 μg per ml inhibited all strains at all inocula. On the reduced growth criterion, all inocula of all 25 strains were inhibited by 0.025 μg per ml.

Thirty-seven strains of Gram-negative bacilli belonging to groups other than *Haemophilus*, and including *Escherichia coli* (13 strains), *Enterobacter aerogenes* (7), *Citrobacter freundii* (1), *Serratia marcescens* (1), *Proteus mirabilis* (6), *Salmonella* serotypes (6) and *Pseudomonas aeruginosa* (3) were all resistant to 1.6 μg per ml of clindamycin, by both criteria, at inocula ranging from undiluted to 10^{-4} .

Clindamycin showed some activity against *H. influenzae*. Out of ten strains tested, the number inhibited completely by 1.6 μg per ml varied from four to nine as the inoculum fell over a 3-log₁₀ range. All nine strains that grew from the smallest inoculum were inhibited by 1.6 μg per ml. In terms of reduction in growth, all ten strains of *H. influenzae* were susceptible to clindamycin 1.6 μg per ml at all inocula tested. At the three smallest inocula, a drug concentration of 0.4 μg per ml was sufficient to reduce growth.

DISCUSSION

Comparison of the MICs of clindamycin and lincomycin for coagulase-positive staphylococci, under equivalent conditions, showed clindamycin to be from 4 to 16 times more active than lincomycin. Very similar relationships were found for coagulase-negative staphylococci. In addition clindamycin was found very active against all strains of β -haemolytic streptococci, *Strep. pneumoniae* and *Strep. viridans*, most of these strains being inhibited by 0.025 μg per ml and the remaining few by 0.05 μg per ml. The concept that clindamycin may be given in lower dosage than lincomycin, thereby reducing the intestinal irritation associated with these drugs, thus derives bacteriological support from this investigation. Resistance of coagulase-positive staphylococci to clindamycin was found very infrequently. Nine of the 300 strains

showed some degree of resistance to the highest drug concentration tested (1.6 μg per ml), although seven of these nine strains showed reduction in growth at this level.

The results with clindamycin are seen in better perspective when compared with the performance of lincomycin, erythromycin and benzylpenicillin against the same 300 strains. Although observations were made for a range of concentrations of each of these drugs, special attention should be paid to the inhibitory activity of critical concentrations of the antibiotics, such as may be attained in the bloodstream after normal dosage. Table II shows that, on these terms, clindamycin gave a better performance than the other three drugs.

Lincomycin is known to be relatively inactive against enterococci. It was not surprising, therefore, to find that clindamycin behaved similarly. However, the median MIC of clindamycin for the three smallest inocula of 50 *Strep. faecalis* strains, was 0.8 μg per ml or less when measured by the reduced growth criterion, although complete inhibition of even the smaller inocula was infrequently achieved even at 1.6 μg per ml. The marked sensitivity of *Strep. faecalis* to erythromycin reinforces the opinion that lincomycin and clindamycin have no place in the treatment of infections due to this organism.

The action of clindamycin on *H. influenzae* is of some interest. Although the inhibitory activity was much less pronounced than that against staphylococci and most streptococci, all ten strains of *H. influenzae* tested against clindamycin in this study showed some degree of inhibition at 1.6 μg per ml.

The inoculum effect with clindamycin, lincomycin and erythromycin was small. An increase in inoculum of coagulase-positive and coagulase-negative staphylococci over a 4-log range did not raise the median MIC more than four-fold. However, the results demonstrate that a change in the inoculum can alter the MIC. Shadomy, Bruce and Kannan (1968) suggest that variations in the conditions of testing account for many of the disagreements concerning the MIC of lincomycin for *Staph. aureus* and the bacteriostatic or bactericidal nature of its effects.

The relative importance of complete inhibition and reduced growth criteria for measurements of MIC remains to be assessed. On theoretical grounds there must be an important difference between a totally resistant population and a predominantly sensitive culture containing a minority of resistant cells. This minority will be of greater or lesser importance clinically according to the rapidity with which it can develop a population large enough to overwhelm the tissue defence. Similar considerations have already been discussed for carbenicillin acting against *Ps. aeruginosa* (Chadwick, 1969).

Clindamycin appears, from these in-vitro studies, to be a very promising antibiotic for the inhibition of staphylococci, pneumococci and streptococci other than enterococci. Its greater activity in comparison with lincomycin should allow its use in considerably smaller dosage, thus reducing considerably the risk of intestinal irritation. No parenteral preparation is yet available, but when one becomes so, clindamycin could possibly supersede lincomycin in treatment of appropriate infections. The choice between clindamycin and a penicillinase-resistant penicillin in the treatment of infections due to penicillin-resistant *Staph. aureus* must remain a matter of judgment in the individual case, but clindamycin would be a powerful agent in the case of such an infection in a patient hypersensitive to penicillin. This investigation suggests that a penicillin-resistant *Staph. aureus* is more likely to be sensitive to clindamycin than to erythromycin. This does not warrant the use of clindamycin without the use of sensitivity measurements; these should be performed to provide bacteriological support for use of the drug, and to detect any trend, especially in hospitals where clindamycin is used, towards development of bacterial resistance.

SUMMARY

The activity of clindamycin, a new lincomycin derivative, was measured against over 500 strains of bacteria. The assessment was quantitative in terms of drug concentration and bacterial population size, and comparative in that the major groups of bacteria were tested simultaneously for their sensitivity to lincomycin, erythromycin, benzylpenicillin and oxacillin. Clindamycin at a low concentration was highly active against most Gram-positive cocci excepting *Strep. faecalis*, and showed appreciable activity against *H. influenzae*, but was

inactive against enterobacteria. Two strains of coagulase-positive staphylococci out of 300 tested were highly resistant to clindamycin; a further seven showed a low degree of resistance. Resistance to lincomycin and erythromycin was considerably commoner. Variation in inoculum size produced a small change in MIC comparable with that found for lincomycin and erythromycin, but much less than that found for benzylpenicillin. On a weight-for-weight basis clindamycin was from 4 to 16 times more active than lincomycin against staphylococci.

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REFERENCES

- CHADWICK, P. 1969. Effect of carbenicillin on *Pseudomonas aeruginosa*. *Canad. Med. Assoc. J.*, **101**, 404.
- DUNCAN, I. B. R., AND JEANS, B. 1965. Lincomycin in hospital practice. *Canad. Med. Assoc. J.*, **93**, 685.
- SHADOMY, S., BRUCE, JUDITH L., AND KANNAN, M. M. 1968. Effect of inoculum size on in vitro susceptibility testing with lincomycin. *Appl. Microbiol.*, **16**, 1663.