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Effect of Clindamycin, Erythromycin, Lincomycin, and Tetracycline on Growth and Extracellular Lipase Production by Propionibacteria In Vitro

SHEILA E. UNKLES AND CURTIS G. GEMMELL*

Department of Bacteriology, Medical School, University of Glasgow, Royal Infirmary, Glasgow, Scotland

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Two propionibacteria identified as *Propionibacterium acnes* and *Propionibacterium granulosum* were grown anaerobically in the presence of growth subinhibitory concentrations (0.25 and 0.5 minimal inhibitory concentrations) of clindamycin, erythromycin, lincomycin, and tetracycline. Viable counts and assays of extracellular lipase were performed on samples taken at 24-h intervals over a 96-h period. The results showed that lincomycin and clindamycin could inhibit the production of the enzyme by both strains with little effect on their growth rates. Tetracycline caused inhibition of lipase production by *P. granulosum* only. Although production of the enzyme by *P. acnes* was delayed in the presence of tetracycline, the final titer was the same as the control. Erythromycin had little effect on growth and enzyme production of either strain. It is possible, therefore, that certain antibiotics used in acne therapy may act not only as bactericidal agents but also as inhibitors of enzyme production under non-growth-limiting conditions.

Hydrolysis of sebum triglycerides by bacterial lipases, in particular those of propionibacteria (13, 20), and the subsequent release of irritating free fatty acids in the pilosebaceous follicles has been proposed as a major factor in the formation of lesions in acne vulgaris (22). Improvement in acne is often achieved by long-term administration of broad-spectrum antibiotics which are thought to decrease the density of propionibacteria (1, 12, 14) and hence reduce the free fatty acids in the sebum (7, 18). At present, there are few reports describing the effects of antibiotics on propionibacteria in vitro, those that do exist being mainly concerned with the evaluation of antimicrobial susceptibility (9, 19). In a recent publication, however, Webster et al. (26) found that lipase production in vitro by a strain of *Propionibacterium acnes* was inhibited by subgrowth-inhibitory concentrations of tetracycline and declomycin and delayed by erythromycin. The present study sought, therefore, to investigate the effect of antibiotics, commonly used in acne therapy, at subinhibitory concentrations on the growth and extracellular lipase production of *P. acnes* and *Propionibacterium granulosum*. Levels of drug at one-half and one-fourth minimal inhibitory concentrations (MIC) were chosen for this study. A similar experimental system has been used to follow the biosynthesis of toxins and enzymes in *Staphylococcus aureus* (5) and *Streptococcus pyogenes* (4).

MATERIALS AND METHODS

Bacterial strains. Two strains (P2 and P27) were selected from a collection of 52 *Propionibacterium* strains which had been isolated from normal skin and maintained anaerobically on Brewer's agar without indicator (Difco Laboratories, Detroit, Mich.), supplemented with 1% Tween 80 (Koch Light, Ltd., Colnbrook, England). The strains were identified using the biochemical classification scheme of Marples and McGinley (15): strain P2 was classified as *P. acnes*, and strain P27 was classified as *P. granulosum*.

Antibiotics. The antibiotics used in this study were obtained from the following sources: clindamycin and lincomycin hydrochlorides, The Upjohn Co., Kalamazoo, Mich.; tetracycline hydrochloride, Lederle Laboratories, Hampshire, England; erythromycin, Abbott Laboratories, Kent, England.

Determination of MIC. Antibiotics were dissolved in 0.85% saline, and the solutions were filter-sterilized through membrane filters (0.45-μm pore size; Millipore Corp., Bedford, Mass.) The solutions were diluted as appropriate in brain heart infusion broth (Gibco-Biocult, Ltd., Paisley, Scotland) supplemented with 0.1% sodium thioglycolate to reduce the Eh.

Suspensions of each strain were made in 0.85% saline from 4-day-old Brewer's agar cultures to give an optical density at 440 nm of 0.70 using a Cecil CE202 spectrophotometer (Cecil Instruments Ltd., Cambridge, England), which was equivalent to a concentration of approximately 3×10^8 colony-forming units (CFU)/ml. One drop (0.02 ml) of bacterial suspension was added to duplicate serial doubling dilutions of each antibiotic in 2 ml of brain heart infusion broth supplemented with 0.1% sodium thioglycolate, giving

an initial viable count of approximately 10^6 CFU/ml of culture medium. Incubation was carried out anaerobically at 37°C for 5 days. The MIC was taken as the highest dilution of antibiotic which inhibited growth.

Growth conditions. Sterile antibiotic solutions were prepared as described above, diluted as appropriate in 0.85% saline, and added to 150 ml of brain heart infusion broth supplemented with 0.1% sodium thioglycolate to give final drug concentrations of one-fourth or one-half MIC for each strain. Suspensions of the two strains were inoculated into the culture medium to give initial viable counts of approximately 3×10^6 CFU/ml. Control cultures containing no antibiotics were also inoculated, and all cultures were incubated at 37°C. Samples were taken at 24-h intervals to determine the viable count (17) and lipase activity. All such determinations were performed in duplicate.

Lipase assay. A semiquantitative method of assay was employed. Culture supernatants were doubly diluted in 0.85% saline and added to 4-mm-diameter wells cut in Columbia agar (Oxoid Ltd., London) containing 1% tributyrin and $1:10^{-6}$ sodium merthiolate (BDH, Poole, England) in glass plates. Although the lipase could hydrolyze triglycerides containing higher fatty acids such as triolein, the assay was easier to read and much more sensitive using tributyrin (glycerol tributyrate) as substrate. One large glass plate (25 cm^2) of substrate agar was used at each sampling time and incubated at 37°C for 48 h. The titer was taken as the reciprocal of the highest dilution of supernatant showing clearing (≥1 mm) around the well. Each supernatant sample was titrated twice to obtain an average titer for further calculations. Good reproducibility between individual titrations was obtained by this method. It was assumed that each well contained 0.04 ml when filled, and the enzyme units per milliliter of sample were calculated accordingly. A subsequent calculation was used to relate the lipase units to colony-forming units of bacteria at various times.

RESULTS

The susceptibilities of both chosen strains (P2 and P27) to each of the four antibiotics were similar, with MIC values of 0.02 μg/ml for clindamycin, 0.08 μg/ml for lincomycin, and 0.02 and 0.01 μg/ml, respectively, for erythromycin. Greater resistance to tetracycline was seen with MIC values of 5.0 μg/ml and 2.5 μg/ml, respectively. From these measurements, subinhibitory concentrations (one-half and one-fourth MIC) of each drug were chosen to study their effect on growth and lipase production.

The growth rate of strain P2 was unaffected by the presence of clindamycin at either one-half or one-fourth MIC (Table 1). Lipase production was reduced for 48 h in the presence of one-fourth MIC and for 72 h in the presence of one-half MIC. On the other hand, clindamycin at one-half MIC inhibited growth of strain P27 up to 72 h. The low amount of lipase detected after 96 h, therefore, probably reflects the inhibition of growth by the antibiotic. The effect on lipase production at one-fourth MIC was more apparent; although growth was not inhibited, lipase production was suppressed for up to 96 h of incubation, and the final value was only half that of the control culture.

Table 2 shows the effect of tetracycline on growth and lipase production by P2 and P27. Little difference was seen in the yield of extracellular lipase by P2 at any point in the 96-h growth period either in the presence or absence of one-half or one-fourth MIC of tetracycline. In contrast, one-half MIC of tetracycline caused impairment of growth and enzyme production in strain P27 for up to 48 h. By 72 h the growth rate compared well with that of the culture grown in the absence of the drug, but no lipase could be detected in the culture supernatant at this time. By 96 h the enzyme yield was considerably less than the control. At the lower drug concentration (one-fourth MIC), there was no clear effect on either growth or lipase production. With strain P27, inhibition of growth with concomitant suppression of extracellular lipase until 48 h was found at one-half MIC. By 72 h, although the viable count had increased, indicating exponential growth, no lipase was detected in the culture supernatant and the maximum value reached at 96 h was considerably less than that of the control. At the lower concentration of one-fourth MIC, lipase production was delayed until 72 h with no apparent effect on growth; by 96 h, enzyme levels were the same in both drug-treated and control cultures.

Erythromycin caused slight inhibition of growth of P2 at the higher concentration (one-half MIC) of the antibiotic, but only until 24 h (Table 3). By 96 h lipase production was marginally higher in the antibiotic-treated cultures than in the control. The drug caused no clear effects on inhibition of growth or lipase production of P27 at either concentration.

Some morphological changes were noted in cultures grown in the presence of the various antibiotics. The control culture with no antibiotic contained very short, gram-positive rods which became gram-variable after 48 h of incubation. When the bacteria were grown in the presence of the higher concentrations (one-half MIC) of antibiotics, the culture was composed mainly of short, gram-positive rods as in the control, but there were also some much longer rods, each up to five times the length of the normal cell. These cells were thickened or bulbous at one end and were not seen in the cultures after 48 h.

DISCUSSION

It is generally agreed that *Propionibacterium* species, which can be readily isolated from both

TABLE 1. Effect of clindamycin on growth and extracellular lipase production by strains P2 and P27

Sampling time (h)	Antibiotic level[a]	P2		P27	
		Viable count (CFU × 10^6)	Lipase (units/CFU)	Viable count (CFU × 10^6)	Lipase (units/CFU)
24	0	94	4.3	138	1.5
	½ MIC	32	0	3	0
	¼ MIC	66	1.5	59	1.7
48	0	594	1.4	264	24.2
	½ MIC	528	0.4	3	0
	¼ MIC	385	2.1	363	2.2
72	0	209	3.8	298	25.8
	½ MIC	125	1.6	3	0
	¼ MIC	190	4.2	429	1.9
96	0	147	5.4	172	37.2
	½ MIC	98	4.0	12	0.9
	¼ MIC	147	5.4	106	15.1

[a] For both strains, ½ MIC = 0.01 μg/ml and ¼ MIC = 0.005 μg/ml.

TABLE 2. Effect of tetracycline on growth and extracellular lipase production by strains P2 and P27

Sampling time (h)	Antibiotic level[a]	P2		P27	
		Viable count (CFU × 10^6)	Lipase (units/CFU)	Viable count (CFU × 10^6)	Lipase (units/CFU)
24	0	178	4.5	135	5.9
	½ MIC	33	3.0	4	0
	¼ MIC	106	7.5	120	0.8
48	0	446	3.6	462	6.9
	½ MIC	170	2.4	8	0
	¼ MIC	528	3.0	594	0.3
72	0	211	7.6	165	9.7
	½ MIC	314	2.5	429	0
	¼ MIC	248	6.4	175	9.1
96	0	127	12.6	83	38.5
	½ MIC	157	10.2	198	2.0
	¼ MIC	112	14.3	82	38.8

[a] Strain P2: ½ MIC = 2.5 μg/ml; ¼ MIC = 1.25 μg/ml. Strain P27: ½ MIC = 1.25 μg/ml; ¼ MIC = 0.625 μg/ml.

pilosebaceous follicles and acne lesions (20), are closely associated with acne vulgaris. Broad-spectrum antibiotics used in acne therapy are thought to decrease the numbers of viable *P. acnes* recovered from lesions and hence reduce the amount of free fatty acids in the sebum (2, 7, 8, 18). In these studies, however, bacteriological sampling methods have been used which do not necessarily represent the organisms of the pilosebaceous duct. Cunliffe et al. (3), using a method by which duct samples were obtained, found no quantitative decrease in the bacterial flora of patients receiving oral tetracycline, although there was a reduction in the free fatty acid content of the sebum.

P. acnes lipase is thought to be of importance in the appearance and development of inflammatory skin lesions due to the release of free fatty acids from sebum triglycerides, which are irritating and comedogenic (10). Recently, it has been shown that lipase itself can act as a chemotactic factor (11) which could be important in the inflammatory process, although it is likely that other chemotactic factors are also involved (21, 23).

Our studies have been directed towards the control of production of this enzyme in vitro. To do so we have made use of certain antibiotics which act on protein biosynthesis by attachment to the bacterial ribosome. Our results have

TABLE 3. Effect of erythromycin on growth and extracellular lipase production by strains P2 and P27

Sampling time (h)	Antibiotic level[a]	P2		P27	
		Viable count (CFU × 10^6)	Lipase (units/CFU)	Viable count (CFU × 10^6)	Lipase (units/CFU)
24	0	178	4.5	135	5.9
	½ MIC	48	4.2	64	6.3
	¼ MIC	140	5.7	178	4.5
48	0	446	3.6	462	6.9
	½ MIC	191	8.2	298	5.4
	¼ MIC	363	4.4	395	4.1
72	0	211	7.6	165	9.7
	½ MIC	152	10.5	198	8.1
	¼ MIC	201	8.0	102	15.7
96	0	127	12.6	83	38.5
	½ MIC	101	15.8	85	37.8
	¼ MIC	108	14.8	90	35.5

[a] Strain P2: ½ MIC = 0.01 μg/ml; ¼ MIC = 0.005 μg/ml. Strain P27: ½ MIC = 0.005 μg/ml; ¼ MIC = 0.0025 μg/ml.

shown that the lincosamine drugs at one-half and one-fourth MIC can inhibit production of lipase. Although only two organisms—one classified as *P. acnes*, the other classified as *P. granulosum*—were used, it is tempting to compare our results in vitro with those of others on the effects of antibiotics in vivo. The results presented here are in accordance with the in vivo studies of Pablo and Fulton (18), who reported that clindamycin dramatically reduced free fatty acids in the sebum of normal volunteers, whereas erythromycin, after causing an initial reduction, proved rather disappointing over a longer period. In this study, erythromycin caused only temporary suppression of lipase production by *P. acnes*, as reported also by Webster et al. (26), and had no effect on *P. granulosum*. It is possible therefore that, in vivo, erythromycin may have a direct bactericidal effect on the bacteria, whereas clindamycin and lincomycin, in addition to any bactericidal effect, may act by suppressing the production of extracellular lipase.

In accordance with the results of Webster et al. (26), tetracycline caused a delay in the production of lipase by *P. acnes*. Lipase production by *P. granulosum* could be almost completely inhibited. *P. granulosum* had been found to be more lipolytic than *P. acnes* (27) and can be isolated with greater frequency from acne patients than from normal subjects (12). It has been suggested, therefore, that *P. granulosum* strains may play a more important role in acne vulgaris (6, 12, 16). The activity of *P. acnes* lipase inhibited directly by tetracycline (24) is at serum concentrations of the drug, as used in therapy, of less than 2 μg/ml (3); this effect will not be seen at the concentration likely to be found in the pilosebaceous follicles themselves. Little is known about the levels of antibiotics found in the pilosebaceous duct, and it is possible that these levels are too low to produce a significant bactericidal effect. It seems more likely that tetracycline reduces the amount of free fatty acids in vivo by inhibiting lipase production.

Our findings suggest that specific antibiotics can alter the physiology of *P. acnes* and *P. granulosum* to such an extent that they are unable to elaborate certain exoproteins (lipase in particular) without affecting their viability. It is likely that other changes occur in the antigenic structure of the cell as already demonstrated in *S. pyogenes* (4). Together such changes undoubtedly will affect the host-parasite interaction, but as yet little is known about the role of such factors in acne vulgaris. In this respect the ability of *P. acnes* and *P. granulosum* to activate complement may become important (25). Levels of antibiotics in the pilosebaceous duct during acne therapy have not been closely monitored but are likely to be lower than those needed to kill the organisms, and hence the physiological response demonstrated in this study in vitro may be responsible for the effect of antibiotics seen in vivo.

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