

Suppression of Polymorphonuclear Leukocyte Chemotactic Factor Production in *Propionibacterium acnes* by Subminimal Inhibitory Concentrations of Tetracycline, Ampicillin, Minocycline, and Erythromycin

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Propionibacterium acnes is the cause of inflammation in acne vulgaris and has been shown to produce potent neutrophil chemoattractants. Different strains of *P. acnes* that were sensitive or resistant to ampicillin, erythromycin, minocycline, and tetracycline were grown in the presence of subminimal inhibitory concentrations of the drugs, and their culture supernatants were assayed for neutrophil chemotactic activity. The presence of subminimal inhibitory concentrations of ampicillin failed to affect chemotactic factor production by any of the strains. Subminimal inhibitory concentrations of tetracycline, minocycline, and erythromycin all produced decreased neutrophil chemotactic activity in *P. acnes* culture supernatants. This inhibition of chemotactic activity was most pronounced in strains of *P. acnes* which were susceptible to the drugs. The addition of antibiotics at appropriate concentrations to control supernatants failed to affect neutrophil migration. The results indicate that subminimal inhibitory concentrations of antibiotics are capable of reducing the inflammatory capacity of *P. acnes*.

Acne vulgaris is a skin disease whose inflammatory component is produced by the host response to *Propionibacterium acnes*. *P. acnes* has been shown to activate complement (10), stimulate lysosomal enzyme release from human neutrophils (11), and produce serum-independent neutrophil chemotactic factors (8, 9).

Antibiotic therapy represents one of the major approaches to the treatment of acne vulgaris. Systemic antibiotics such as tetracycline and erythromycin have been the agents of choice for more than 24 years. More recently topical antibiotic formulations including tetracycline, erythromycin, and clindamycin have been widely used. The primary mode of action for these agents has been viewed to be a direct antibacterial effect on the anaerobic diphtheroid *P. acnes*. More recently, attention has been drawn to possible anti-inflammatory effects of antibiotics. Plewig and Shopf (7), for example, demonstrated that systemic and topical antibiotics can suppress the induction of the sterile pustules which are produced under occlusive dressings of potassium iodide. This finding, coupled with the in-vitro demonstration that tetracycline will inhibit neutrophil chemotaxis (2, 5), has fostered the concept that antibiotics may affect acne by means other than direct antibacterial effects.

Previously, we have shown that concentra-

tions of erythromycin and tetracycline which do not inhibit the growth of *P. acnes* can suppress the production of extracellular lipase (12). A similar phenomenon has been reported for lipase (6), hemolysin (3), and DNase (3) production in *Staphylococcus aureus*. A possible explanation for these results is that tetracycline produced a generalized inhibition of protein synthesis, thereby suppressing production of these extracellular enzymes.

In this study we investigated the effect of subminimal inhibitory concentrations (sub-MIC) of tetracyclines and erythromycin on the production of neutrophil chemotactic factors by *P. acnes*. These factors have been shown to be low-molecular-weight peptides which are presumably derived from postsynthetic protein processing (8, 9). We will show that concentrations of antibiotic which fail to alter *P. acnes* growth produce a significant suppression of chemotactic factor production.

MATERIALS AND METHODS

P. acnes American Type Culture Collection 6919, Virginia Polytechnic Institute 3706 (obtained from C. S. Cummins), DH1, and DH6 were grown anaerobically under N₂-CO₂ in CMRL 1066 (GIBCO Laboratories) at 37°C (9).

MIC of tetracycline, erythromycin, minocycline,

TABLE 1. Antibiotic MIC for *P. acnes* strains

Strain	MIC ($\mu\text{g/ml}$)			
	Ampicillin	Tetracycline	Minocycline	Erythromycin
6916	0.125	0.625	0.313	0.625
3706	0.125	0.625	0.313	0.313
DH1	0.125	5.0	5.0	>10.0
DH6	0.125	10.0	5.0	10.0

and ampicillin for all strains were determined by broth dilution in CMRL 1066. Sub-MIC of antibiotics were added to appropriate cultures at the time of *P. acnes* inoculation. Before chemotaxis assay, culture supernatants were spun free of cells, filtered through a 0.45- μm membrane filter (Millipore Corp.), and adjusted to pH 7.0.

Chemotaxis. Human neutrophils were obtained from the buffy coat of heparinized human blood washed and adjusted to 2.5×10^6 neutrophils per ml in CMRL 1066 with 10% fetal calf serum. The chemotaxis assay was performed in a Sykes-Moore chamber with a nitrocellulose filter as previously described (9, 10). The number of polymorphonuclear leukocytes in 10 high-power fields of the leading front of cells was counted and termed the chemotactic index. Chemotactic activity was expressed as the chemotactic index divided by the optical density of the culture which produced the chemotactic supernatant. This served to normalize the chemotactic indices to correct for any subtle variations in growth. The mean chemotactic responses to treated and untreated cultures were used to calculate the percent inhibition of chemotactic factor production.

RESULTS

MIC or tetracycline, erythromycin, minocycline, and ampicillin were determined for *P. acnes* strains 6919, 3706, DH1, and DH6 in CMRL 1066 medium (Table 1).

Spectrophotometrically monitored growth curves were performed in the presence of sub-MIC of the drugs. Cultures containing the highest concentration of antibiotic which did not affect the growth curve were tested for chemotactic activity. Sample growth curves are presented in Fig. 1.

Antibiotics at appropriate concentrations were added to control, untreated *P. acnes* supernatants and failed to affect the polymorphonuclear leukocyte migratory response.

P. acnes 6919 displayed 31% inhibition by 0.05 μg of tetracycline per ml ($P < 0.01$), a 21% inhibition by 0.05 μg of minocycline per ml ($P < 0.05$), and a 22% inhibition by 0.05 μg of erythromycin per ml ($P < 0.05$). *P. acnes* 3706 behaved in a similar fashion, displaying a 40% inhibition in chemotactic factor production by 0.05 μg of tetracycline per ml ($P < 0.001$), a 37% inhibition by 0.01 μg of minocycline per ml ($P < 0.01$), and a 16% inhibition by 0.01 μg of erythromycin per

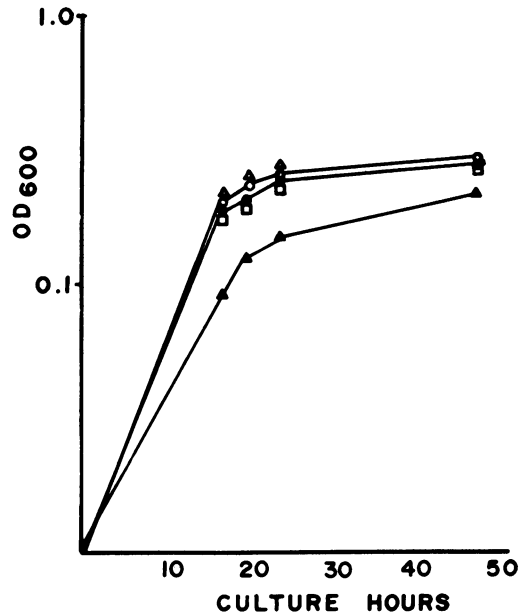


FIG. 1. Growth curve of *P. acnes* 6919 in the presence of sub-MIC of antibiotics. Symbols: \circ , no antibiotics; \triangle , 0.05 μg of tetracycline per ml; \bullet , 0.05 μg of erythromycin per ml; \square , 0.01 μg of ampicillin per ml; \blacktriangle , 0.05 μg of minocycline per ml.

ml (not significant). Neither strain displayed any inhibition in chemotactic factor production by ampicillin.

P. acnes DH1 and DH6, which were resistant to tetracycline and erythromycin, were less inhibited in chemotactic factor production by sub-MIC of the drugs. DH1 displayed no inhibition by 0.01 μg of ampicillin per ml, 14% inhibition by 0.5 μg of tetracycline per ml, 6% inhibition by 0.5 μg of erythromycin per ml, and 13% inhibition by 0.5 μg of minocycline per ml (all not significant). DH6 displayed no inhibition in chemotactic factor production by 0.01 μg of ampicillin per ml, 12% inhibition by 0.5 μg of tetracycline per ml, 15% by 0.5 μg of erythromycin per ml ($P < 0.05$), and 10% inhibition by 0.5 μg of minocycline per ml (not significant) (Table 2).

DISCUSSION

To date, almost all reports of the effects of sub-MIC of antibiotics on bacteria have dealt with structural or morphological changes after exposure to drugs. Numerous papers (reviewed in reference 4) reported that sub-MIC of antibiotics induce distorted cell structure in bacteria. Alkan and Beachy (1) have demonstrated that sublethal concentrations of penicillin impair the ability of streptococci and *Escherichia coli* to adhere to epithelial cells. Lorian (4) has reported that sub-MIC of tetracycline render bacteria

TABLE 2. Inhibition of chemotactic factor production of *P. acnes* strains by sub-MIC antibiotics^a

Strain	Resistance	Chemotactic index/optical density (SD)				
		Control	AM	TC	MN	EM
6919		510 (27)	506 (33)	351 (30)	403 (51)	397 (49)
3706		566 (37)	571 (23)	339 (43)	357 (22)	475 (48)
DH1	TC, MN, EM	570 (45)	575 (25)	490 (59)	496 (48)	536 (31)
DH6	TC, MN, EM	552 (29)	548 (57)	486 (47)	497 (38)	469 (23)

^a Abbreviations: AM, ampicillin; TC, tetracycline; MN, minocycline; EM, erythromycin.

more susceptible to killing by heat. In this paper we have reported that sub-MIC of tetracyclines and erythromycin can inhibit the production of neutrophil chemotactic factors in susceptible strains of *P. acnes*. With one exception, erythromycin and strain 3706, this was a statistically significant inhibition. Resistant strains also displayed decreased chemotactic factor production in the presence of sub-MIC of antibiotics, but to a much lesser degree. The data do not permit conclusions regarding the relative clinical efficacy of the drugs tested.

Tetracycline, minocycline, and erythromycin all inhibit bacterial protein synthesis. Since the chemotactic factor of *P. acnes* is a low-molecular-weight peptide, presumably the product of postsynthetic protein processing, the data may be interpreted to reflect an inhibition of protein synthesis by sub-MIC of antibiotics. The fact that ampicillin, a drug whose action is directed against the cell wall, fails to inhibit chemotactic factor production is consistent with this interpretation.

The inhibition of chemotactic factor production by antibiotics may account for the anti-inflammatory effects of tetracycline and erythromycin in acne vulgaris in a more attractive way than does a generalized inhibition of neutrophil mobility. This observation may also suggest a new role for antibiotics as adjunctive therapy in atopic dermatitis, periodontitis, and other diseases whose severity is increased by bacterial colonization of affected areas. A reduction in the inflammatory capacity of the colonizing organisms could enhance clinical improvement.

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LITERATURE CITED

1. Alkan, M. C., and E. H. Beachy. 1978. Excretion of lipoteichoic acid by group A streptococci. Influence of penicillin on excretion and loss of ability to adhere to human oral mucosal cells. *J. Clin. Invest.* **61**:671-684.
2. Goodhart, G. L. 1979. Further evidence for the role of bivalent cations in human polymorphonuclear leukocyte locomotion, recovery from tetracycline-induced inhibition in the presence of cation ionophores. *RES J. Reticuloendothelial Soc.* **25**:545-556.
3. Hinton, N. A., and J. H. Orr. 1960. The effect of antibiotics on the toxin production of *Staphylococcus aureus*. *Antibiot. Chemother.* **10**:758-767.
4. Lorian, V. 1980. Antibiotics in laboratory medicine, p. 343-409. The Williams & Wilkins Co., Baltimore.
5. Martin, R. R., G. A. Warr, R. B. Couch, L. T. Yeager, and V. Knight. 1974. Effects of tetracycline on leukotaxis. *J. Infect. Dis.* **129**:110-116.
6. Mates, A. 1973. Inhibition by tetracycline in the occurrence of extracellular lipase from *Staphylococcus aureus*. *Microbios* **7**:25-32.
7. Plewig, G., and E. Shopf. 1975. Anti inflammatory effects of antimicrobial agents. *J. Invest. Dermatol.* **65**:532-536.
8. Puhvel, S. M., and M. I. Sakamoto. 1978. The chemottractant properties of comedonal components. *J. Invest. Dermatol.* **71**:324-329.
9. Webster, G. F., and J. J. Leyden. 1980. Production of serum-independent polymorphonuclear leukocyte chemotactic factors by *Propionibacterium acnes*. *Inflammation* **4**:261-269.
10. Webster, G. F., J. J. Leyden, M. E. Norman, and U. R. Nilsson. 1978. Complement activation in acne vulgaris. In vitro studies with *Propionibacterium acnes* and *Propionibacterium granulosum*. *Infect. Immun.* **22**:523-529.
11. Webster, G. F., J. J. Leyden, C.-C. Tsai, P. Baehni, and W. P. McArthur. 1980. Polymorphonuclear leukocyte lysosomal release in response to *Propionibacterium acnes* in-vitro and its enhancement by sera from inflammatory acne patients. *J. Invest. Dermatol.* **74**:398-401.
12. Webster, G. F., K. J. McGinley, and J. J. Leyden. 1981. Inhibition of lipase production in *Propionibacterium acnes* by sub-minimal inhibitory concentrations of tetracycline and erythromycin. *Br. J. Dermatol.* **104**:453-457.