The Effects of Tetracyclines and Erythromycin on Complement Activation In vitro

E. INGHAM

Department of Immunology, University of Leeds, Leeds, W. Yorkshire, England

The effects of tetracycline, minocycline and erythromycin on complement activation in vitro were studied. At concentrations of 100 mg/l or less, these antibiotics did not inhibit the capacity of *Propionibacterium acnes* to cleave C3 in normal human serum or in serum chelated of Ca^{2+} allowing complement activation by the alternative pathway alone. The antibiotics had no effect (at 100 mg/l) on total haemolytic activity of complement in normal human serum. This study did not provide evidence to support the hypothesis that the efficacy of these antibiotics in the therapy of inflammatory acne vulgaris can be explained by inhibition of complement activation.

(Accepted April 9, 1990.)

Acta Derm Venereol (Stockh) 1990; 70: 531–534.

E. Ingham, Department of Immunology, The University of Leeds, The General Infirmary, Leeds LS1 3EX, Great Britain.

Tetracycline and erythromycin antibiotics have been used for many years in the treatment of acne vulgaris. However, their mode of action in this disease is not fully understood. It has been suggested that antibacterial activity alone is not sufficient to explain their beneficial effects and that interaction with host defence mechanisms may contribute to their efficacy (1). One of the earliest histologically-apparent signs of inflammation in acne lesions is the deposition of complement C3 at the basement membrane zone of the majority of comedones and in the walls of adjacent dermal blood vessels (2, 3). The demonstration of C3, in the absence of immunoglobulins and C1q, has been interpreted as alternative pathway activation of complement (2, 3). The contents of acne lesions have been shown to activate complement by the classical pathway (4) and individually expressed comedones have been shown to activate complement by the alternative pathway in vitro (5). The extent of complement activation has been shown to correlate with numbers of *Propionibacterium acnes* present in comedonal extracts (5).

The tetracycline antibiotics have been shown to depress the bacteriocidal activity of human serum (6). It has been suggested that this effect was due to impairment of complement activation by the alternative pathway (6). In view of the possibility that the efficacy of these antibiotics in acne therapy might be explained, at least in part, by inhibition of complement activation, this study was undertaken to test the effects of tetracycline, minocycline and erythromycin on complement activation in vitro.

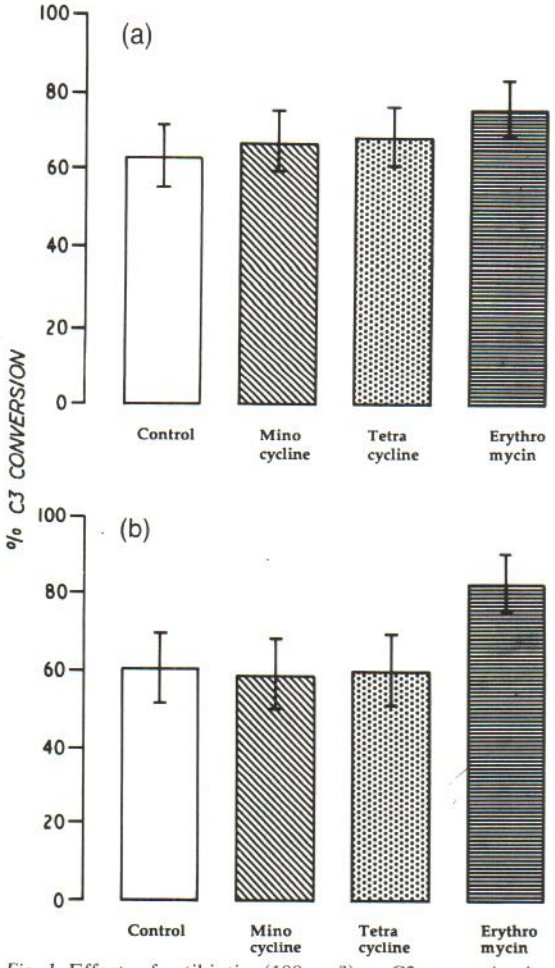

Fig. 1. Effects of antibiotics (100 mg/l) on C3 conversion by *P. acnes* in normal human serum. (*a*) Unchelated serum, analysis of variance demonstrated no significant effects of antibiotics. (*b*) Serum chelated of Ca^{2+}, allowing alternative pathway activation only. Analysis of variance demonstrated a significant effect amongst the antibiotics ($p < 0.05$). Analyses were carried out on data subjected to angular transformation ($\sin^{-1}\sqrt{\text{proportion}}$). Data was back-transformed to percentages before being plotted graphically. Results are expressed as means ($n = 6$) ± 1/2 L.S.D. ($p < 0.05$). Error bars attached to tests (with antibiotics) which do not overlap with error bars attached to the control denote significant difference.

MATERIALS AND METHODS

Serum samples

Samples of blood were drawn by venepuncture from 6 normal adults. The blood was allowed to clot at 37°C for 1 h and the serum obtained by centrifugation. Sera were used immediately or stored at −70°C until use.

P. acnes for complement activation

P. acnes (strain P-37; type I; isolated from a blackhead lesion) was grown in Brain Heart Infusion broth (Difco) plus glucose (3 g/l) for 72 h in static batch culture in an atmosphere of H_2/CO_2(90 : 10; v/v) at 37°C. *P. acnes* were harvested by centrifugation, washed and resuspended at 10^{10} cells/ml in phosphate-buffered saline (PBS; pH 7.4). The cells were then formalized (1% v/v formalin in PBS for 24 h), washed a further three times in PBS and resuspended in PBS at 10^{10} cells/ml.

Antibiotics

Fresh stock solutions of tetracycline HCl, minocycline HCl and erythromycin (all obtained from Sigma) were prepared by dissolving the antibiotics in methanol (BDH, analar) at 10 g/l. Immediately prior to use the stock antibiotics were diluted 1 : 10 in PBS to give the desired final concentration.

Effects of antibiotics on the capacity of P. acnes *to cleave C3 in normal human serum*

Duplicate serum samples (100 µl) were incubated with *P. acnes* (20 µl; 10^{10}/ml) and the antibiotic (15 µl) or PBS (15 µl) at 37°C for one hour. In one series of experiments, 0.2 M-EGTA [ethyleneglycol *bis*-(aminoethyl)-tetra acetic acid; 15 µl in PBS] plus 0.3 M Mg_2SO_4 (15 µl in PBS) was included to allow complement activation by the alternative pathway only. In a second series of experiments, 30 µl of PBS was added, allowing complement activation to proceed via both the classical and alternative pathways. At time zero and after one hour at 37°C, 10 µl of 0.2 M EDTA (ethylenediamine-tetra acetic acid; Sigma) was added to 75-µl aliquots of each reaction mixture to arrest C3 cleavage by either pathway. The aliquots were frozen at −70°C until assayed for C3 cleavage by standard 2-dimensional electrophoresis as described previously (7). The extent of C3 cleavage was calculated as the percentage of total C3 (β1C plus β1A peaks) migrating as cleavage product (β1A peak) at one hour, minus the percentage C3 cleavage at time zero.

Effects of antibiotics on total haemolytic complement activity of serum, using a 50% haemolytic end-point

Serum (450 µl) was incubated with 50 µl of each antibiotic (1 g/l; 5 µl of a 10 g/l solution in methanol plus 45 µl PBS) or 50 µl diluent (45 µl PBS plus 5 µl methanol) for one hour at 37°C and then immediately frozen at −70°C, until assayed for total haemolytic complement.

C'H50 determinations were carried out in duplicate in Isogevers buffer (complement fixation test buffer, Oxoid Ltd. plus 10% w/v gelatin) using conventional methodology (8). The test sera (with antibiotics) were assayed in Isogevers buffer containing 100 mg/l of the appropriate antibiotic, control sera, in absence of antibiotics. Briefly, freshly sensitized sheep red blood cells (S-SRBC; 1 ml; 5×10^8, Gibco) were added to each of six appropriate dilutions of the test and control sera (6.5 ml) in glass tubes. Total lysis and spontaneous lysis controls were included. After 90 min at 37°C, tubes were centrifuged (2000 *g*; 10 min) and the optical density (541 nm) of the supernatants determined. The 50% haemolytic end-point for each test and control serum was determined by dividing the OD 541 nm of each

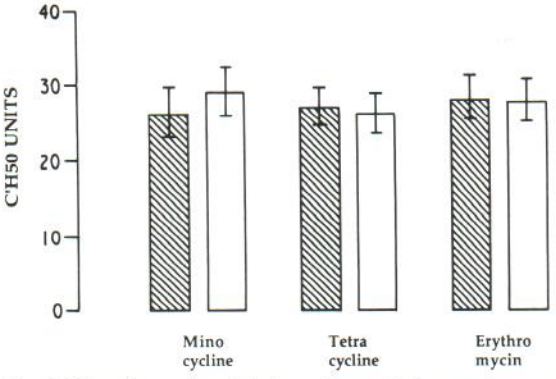

Fig. 2. The effects of antibiotics on haemolytic complement activity (C'H50) in normal human serum. □, Normal human serum; ▨, normal human serum plus 100 mg/l antibiotic. Error bars represent means ($n = 6$) ± 95% confidence limits. Analysis of the data (paired *t*-tests) revealed no significant effects of the antibiotics.

dilution by the OD 541 nm of the total lysis control in order to ascertain the percentage lysis followed by Probit analysis. Using this method, one C'H50 unit was defined as that quantity of complement which would lyse 2.5×10^8 optimally sensitized SRBC out of a total of 5×10^8 S-SRBC, in 90 min at 37°C in a total volume of 7.5 ml.

Statistical methods

Paired *t*-tests and analysis of variance were performed according to the recommendations of Sokal & Rohlf (9).

RESULTS

Effects of antibiotics on the capacity of P. acnes *to cleave C3 in normal human serum*

In preliminary experiments, tetracycline, minocycline and erythromycin at final concentrations of 0.1, 0.5, 1, 5, 10 and 100 mg/l were tested in two serum samples for an effect on C3 cleavage by *P. acnes*. The antibiotics did not affect the percentage C3 cleaved by the alternative pathway alone or the percentage C3 cleaved in unchelated serum. Further studies were carried out with 100 mg/l antibiotics in six normal human serum samples. The results are shown in Fig. 1. The results demonstrated that neither tetracycline, minocycline, nor erythromycin affected the capacity of *P. acnes* to cleave complement C3 in the six samples of normal human serum. In serum chelated of Ca^{2+}, to allow the alternative pathway of complement only to be activated, two-way analysis of variance revealed a significant effect amongst the antibiotics ($p < 0.05$). when the means of percentage C3 cleavage with each antibiotic were compared with the control, using Least Significant Difference [L.S.D. as recommended by Sokal & Rohlf (9)], it was demonstrated that erythromycin at 100 mg/l significantly enhanced C3 cleavage by *P. acnes* via the alternative pathway ($p < 0.05$).

Effects of antibiotics on total haemolytic complement activity of serum

In order to determine whether the antibiotics affected the functional activity of complement, six normal human sera were incubated with and without minocycline, tetracycline and erythromycin at 100 mg/l and then assayed for C'H 50 in the presence and absence of the antibiotics. The results are shown in Fig. 2. This data were analysed using paired *t*-tests. There was no significant effect of the antibiotics on the total haemolytic complement activity in normal human serum.

DISCUSSION

Cleavage of the major complement protein, C3, is central to both the classical (Ca^{2+} dependent) and alternative (Mg^{2+} dependent) pathways of complement activation. Tetracycline, minocycline and erythromycin at above the therapeutic concentration range failed to affect C3 cleavage by *P. acnes* in unchelated serum.

At 100 mg/l, erythromycin significantly enhanced C3 cleavage by *P. acnes* by the alternative pathway. This affect was not, however, apparent in therapeutic concentration ranges (less than 10 mg/l). The mechanism of action of erythromycin in this respect is not known. However, since formalized *P. acnes* cells were used in these studies, a direct effect on the lability of C3 in serum would seem likely. This finding may have implications for the use of topical erythromycin therapy. All three antibiotics failed to significantly affect the functional capacity of complement to lyse sensitized sheep red blood cells at supra-physiological concentrations.

Forsgren & Gnarpe (6) concluded, from data obtained using bactericidal tests, that the tetracyclines inhibited the alternative pathway of complement activation in serum due to chelation of Mg^{2+} ions. Two observations are pertinent to these apparently contradictory findings. Firstly, Forsgren & Gnarpe (6) did not observe a decrease in the opsonic capacity of serum in the presence of tetracyclines, indicating no adverse effect on the early acting (to C3b) alternative complement pathway. Secondly, the mechanisms by which complement kills nucleated cells

(e.g. *Gram -ve* bacteria) are poorly understood and are more complex than those involved in the lysis of erythrocytes (10, 11). It is possible that the tetracyclines impair complement-mediated killing of serum sensitive bacteria by affecting late-acting complement proteins [for example, formation of oligomeric C9, not required for the lysis of erythrocytes (11)] and not by inhibiting alternative pathway activation *per se*. It has been suggested that the chelating properties of tetracyclines might interfere with complement activation by affecting the concentrations of Ca^{2+} and Mg^{2+} (6). Since no inhibition of complement activation by the tetracyclines was observed in these studies, even at a concentration of 100 mg/l, it is unlikely that such effects accounted for the different results obtained for the tetracyclines as compared with erythromycin.

With regard to the effects of these antibiotics in the treatment of inflammatory acne, it is relevant that these drugs did not inhibit the cleavage of C3 by *P. acnes*. Thus, this study has not provided evidence that the efficacy of these antibiotics in inflammatory acne can be explained by their effects on complement activation. Since the tetracyclines and erythromycin have been shown to inhibit lymphocyte transformation in vitro (12, 13) and depress phagocyte functions (14) these non-antimicrobial effects may be important in their efficacy in the treatment of inflammatory acne.

REFERENCES

1. Eady EA, Holland KT, Cunliffe WJ. The use of antibiotics in acne therapy: oral or topical administration? J Antimicrob Chemother 1982; 10: 89–115.
2. Scott DG, Cunliffe WJ, Gowland G. Activation of complement – a mechanism for the inflammation in acne. Br J Dermatol 1979; 101: 315–320.
3. Dahl M, McGibbon D. Complement C3 and immunoglobulin in inflammatory acne vulgaris. Br J Dermatol 1979; 101: 633–640.
4. Webster GF, Leyden JJ, Nilsson UR. Complement activation in acne vulgaris: consumption of complement by comedones. Infect Immun 1979; 26: 183–186.
5. Leeming JP, Ingham E, Cunliffe WJ. The microbial content and complement C3 cleaving capacity of comedones in acne vulgaris. Acta Derm Venereol (Stockh) 1988; 68: 468–473.
6. Forsgren A, Gnarpe H. Tetracyclines and host defence mechanisms. Antiamicrob Agents Chemother 1973; 3: 711–715.
7. Ingham E, Gowland G, Taylor K. Stabilisation of C3 in EDTA-plasma at pH 6.0. Med Lab Sci 1985; 42: 30–34.
8. Mayer MM. Complement and complement fixation. In: Kabat EA, Ed. Experimental Immunochemistry, 2nd edn. Illinois, U.S.A.: Charles C Thomas, 1967.
9. Sokal R, Rohlf F. Single classification analysis of variance and paired comparisons. In: Biometry, chapters 9 & 11. San Francisco: WH Saunders, 1981: 219–242, 359.
10. Kim SH, Carney DF, Hammer CH, Shin ML. Nucleated cell killing by complement. Effects of C5b-9 channel size and extracellular Ca^{2+} on the lytic process. J Immunol 1987; 138: 1520–1536.
11. Bloch EF, Schmetz MA, Foulds J, Hammer CH, Frank MM, Joiner KA. Multimetric C9 within C5b-9 is required for inner membrane damage to *Eschericia coli* J5 during complement killing. J Immunol 1987; 138: 842–848.
12. Forsgren A, Banck G. Influence of antibiotics on lymphocyte function in vitro. Infection 1978; 6: 91–97.
13. Munster AM, Loadholdt CB, Leary AG, Barnes MA. The effects of antibiotics on cell-mediated immunity. Surgery 1977; 81: 692–695.
14. Esterley NB, Furey NL, Flanagan LE. The effect of antimicrobial agents on leucocyte chemotaxis. J Invest Dermatol 1978; 70: 51–55.