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In vivo and *in vitro* effects of doxycycline on leucocyte membrane receptors

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SUMMARY

Tetracyclines, particularly doxycycline, have adverse effects on granulocyte function *in vitro*. We have examined the effects of doxycycline on membrane receptors for IgG (Fcγ-R) and C3b (C3b-R) on granulocytes and lymphocytes, as well as on the sheep erythrocyte receptor (E-R) on T lymphocytes. Acne patients given doxycycline orally had a lower percentage of Fcγ-R positive granulocytes (57%) than before treatment (80%) or compared to healthy controls (81%). Following *in vitro* doxycycline incubation, normal granulocytes showed decreased levels of Fcγ-R positive cells. This effect was counteracted by the addition of magnesium during incubation. The deleterious effect of doxycycline on granulocyte functions may be due to decreased levels of Fcγ-R bearing granulocytes. Doxycycline *in vivo* or *in vitro* had no significant effect on the proportion of C3b-R bearing granulocytes or lymphocytes or the T lymphocyte percentage. After *in vitro* irradiation with light at 340–380 nm, however, both granulocytes and lymphocytes preincubated with doxycycline showed up to 50% decrease in Fcγ-R bearing cells, while control cells without doxycycline were unaffected.

Keywords doxycycline Fc receptor C3b receptor leucocytes

INTRODUCTION

The effects of tetracyclines, and in particular doxycycline, on polymorphonuclear leucocyte (PMN) functions have been extensively studied. *In vitro* studies have shown that doxycycline has deleterious effects on PMN migration (Belsheim, Gnarpe & Løfberg, 1979; Glette *et al.*, 1982), phagocytosis (Forsgren, Schmeling & Quie, 1974; Gnarpe & Leslie, 1974), chemiluminescence and glucose oxidation (Glette *et al.*, 1982). The results of some, but not all, *in vivo* studies also indicate a deleterious effect (Glette *et al.*, 1982). In addition, Forsgren & Gnarpe (1973) found doxycycline to inactivate the complement system and interfere with the bactericidal effect of serum. Little is known about the mechanisms underlying these various effects of doxycycline. In the present study doxycycline *in vivo* and *in vitro* is shown to depress rosette formation between PMNs and antibody-coated erythrocytes, thus implying interference with the receptor for the Fc portion of immunoglobulin G.

MATERIALS AND METHODS

Peripheral blood was obtained from eight patients with acne, and from 25 healthy controls. Samples from the patients were taken immediately before and 1 week after institution of 200 mg doxycycline orally per day. PMNs and lymphocytes were separated as described by Talstad (1981). Briefly, 2 ml

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of heparinized (18 u/ml) blood was mixed with 2 ml of phosphate-buffered saline (PBS) and carefully layered on top of a two-layer mixture of Ficoll (Pharmacia, Stockholm, Sweden) and Isopaque (Nyegaard & Co., Oslo, Norway) in a plastic tube. After centrifugation at 600 ***g*** for 30 min the PMNs and lymphocytes, forming separate layers, were harvested, washed three times in PBS and resuspended in PBS to a concentration of 4×10^6/ml.

Rosette techniques. 'Active' E-rosette-forming cells (active E-RFC) were enumerated by adding 0·2 ml of 1% sheep erythrocytes (E) to 0·2 ml of lymphocyte suspension, centrifuging at 200 ***g*** for 5 min, resuspending, and counting (Smith *et al.*, 1975). The active E-RFC population is considered to represent the immunologically active T lymphocytes (Wybran & Fudenberg, 1973).

E-rosette-forming cells (E-RFC) were determined as described previously (Næss & Nyland, 1978). A lymphocyte suspension of 0·2 ml was mixed with an equal volume of 0·5 E, incubated for 5 min at 37°C, centrifuged for 5 min at 200 ***g***, and stored in ice water overnight before being counted.

PMN suspensions did not form active E-RFC or E-RFC.

EA-rosette-forming cells (EA-RFC) were enumerated by an assay with ox E sensitized with $\frac{1}{2}$ agglutinating unit of rabbit IgG antibodies (A). By this technique the presence on the cell of receptors for the Fc portion of IgG (Fcγ-R) is demonstrated (Hallberg, Gurner & Coombs, 1973). Lymphocytes or PMN were mixed with an equal volume (0·2 ml) of indicator cells, and incubated at room temperature for 20 min.

EAC-rosette-forming cells (EAC-RFC) were determined using sheep E sensitized with rabbit IgM antibodies (A). Human serum absorbed with zymosan was used as the source of complement (C). A mixture of 0·2 ml of lymphocyte or PMN suspension and 0·2 ml of EAC was incubated at 37°C for 20 min. Cells having receptors for the C3b fraction of complement (C3b-R) form rosettes by this method (Matre & Tønder, 1976).

All rosette preparations were counted by the same person, at a magnification of ×600. A minimum of 200 cells were counted and those with four or more adhering E were counted as RFC.

Incubation studies. Samples of 0·2 ml of PMN or lymphocytes were incubated at 37°C for 30 min in PBS, or PBS with doxycycline at the concentration of 6·25, 12·5, 25, 50 and 100 μg/ml. After incubation, cells were washed three times in PBS, and resuspended to 4×10^6/ml. Rosette studies were then performed as described above. In an additional experiment, 4 mM of Mg^{2+} was added to incubation tubes containing 50 μg doxycycline/ml. Similar experiments using Ca^{2+} were abandoned because of excessive clumping of cells.

Irradiation. Contaminating erythrocytes in the granulocyte suspension were first lysed with ammonium chloride (Weening, Roos & Loos, 1974). The cells were than resuspended in PBS, pH 7·3, to a concentration of 7×10^6 cells/ml. Granulocytes and lymphocytes were incubated for 15 min at 37°C in PBS or PBS with 10 μg/ml of doxycycline. Irradiation was carried out at 20°C in 10-mm diameter test tubes, using a photochemotherapy unit PUVA (H. Haldmann, D-722 Schwenningen, FRG) containing 14 fluorescent tubes (F8T5/BL PUVA, Sylvania) in a bank. About 70% of the emission energy of these lamps is between 340 and 380 nm. The light intensity is 66 W/m² at sample level as measured with a UDT model ×80 optometer equipped with a radiometric filter (United Detector Technology, Inc., Santa Monica, California, USA). After 30 min irradiation the cells were centrifuged, resuspended in PBS to a concentration of 4×10^6 cells/ml, and the EA-RFC test was performed.

Statistics. The statistical significance of the observed differences was established by Wilcoxon tests on unpaired and paired samples as appropriate.

RESULTS

The pre-treatment levels of Fcγ-R and C3b-R bearing PMN or lymphocytes from acne patients were not significantly different from those of the controls (Table 1). During treatment, however, PMN Fcγ-R percentages decreased to a mean of 57% ($P < 0{\cdot}05$). Lymphocyte Fcγ-R levels were not significantly affected, nor were the proportions of T lymphocytes (E-RFC). The percentage of C3b-R bearing cells did not differ from controls.

Incubation of PMN in increasing concentrations of doxycycline resulted in a significant decrease in PMN Fcγ-R bearing cell percentage (Fig. 1). The levels of Fcγ-R bearing lymphocytes also decreased during incubation, but less markedly.

Table 1. Effect of oral doxycycline treatment on leucocyte membrane receptors

		Receptor-bearing granulocytes (%)		Receptor-bearing lymphocytes (%)			
	No.	Fcγ	C3b	E active	E	Fcγ	C3b
Acne patients before treatment	8	80±7	69±28	34±12	67±14	32±17	38±12
Ance patients during treatment	8	57±30*	80±6	39±13	61±17	28±18	43±9
Controls	25	81±12	74±17	39±12	66±11	29±9	35±12

Results are mean values±s.d.
*$P<0.05$

When PMN or lymphocytes were incubated in 50 μg doxycycline/ml there was a significant decrease of Fcγ-R bearing PMN which was counteracted when 4 mM Mg^{2+} was included in the medium (Table 2).

Incubation in 50 μg doxycycline/ml with or without 4 mM Mg^{2+} had no significant effect on the Fcγ-R bearing lymphocyte levels.

After PUVA irradiation, granulocytes and lymphocytes preincubated in 10 μg doxycycline/ml showed a significant decrease in Fcγ-R bearing cell percentage, which was not observed in cells not pre-incubated with doxycycline (Table 3).

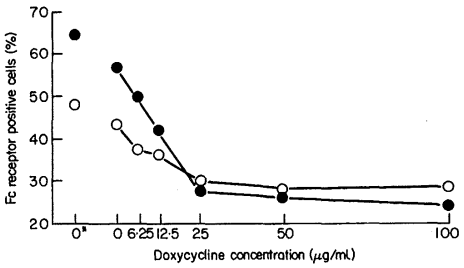

Fig. 1. Percentage of Fc receptor positive cells after incubation of granulocytes (●) and lymphocytes (○) in PBS with and without doxycycline. * = No incubation.

Table 2. *In vitro* effect of doxycycline on leucocyte Fcγ receptors

	Fcγ receptor bearing	
	Granulocytes (%)	Lymphocytes (%)
Incubation		
None	74±9	44±18
PBS	65±13*	39±14
Doxycycline 50 μg/ml	45±16*†	37±10
Doxycycline 50 μg/ml with 4 mmol Mg^{2+}	65±13†	47±16

Results are mean of six experiments±s.d.
*$P<0.01$
†$P<0.01$

Table 3. Percentages of Fcγ-R bearing cells before and after exposure to PUVA light

		After irradiation (30 min)	
	Before irradiation	Doxycycline$^+$	Doxycycline$^-$
Granulocytes	57±8	30±10	64±17
Lymphocytes	33±9	20±15	30±6

Doxycycline$^+$ after 15 min preincubation at 37°C in doxycycline (10 μg/ml) in PBS.

Doxycycline$^-$ after 15 min preincubation at 37°C in PBS without doxycycline.

Results are mean±s.d. (five experiments).

DISCUSSION

We have shown that doxycycline *in vitro* or *in vivo* is associated with a decrease in the proportion of Fcγ-R bearing PMN. Untreated acne patients had normal levels of C3b-R and Fcγ-R bearing cells, which is consistent with the findings of Rebora, Dallegri & Patrone (1979) of normal neutrophil functions in such patients. Previous studies by several authors have documented a deleterious effect of tetracyclines on several PMN functions (Belsheim *et al.*, 1979; Forsgren *et al.*, 1974; Glette *et al.*, 1982). The Fcγ-R is important in the process of phagocytosis (Kelmpner & Gallin, 1978), and interference with this receptor may explain the impaired capacity of doxycycline-incubated PMN to phagocytize (Forsgren *et al.*, 1974). One would expect the effect of doxycycline on the Fcγ-R of the PMN to be paralleled by similar results regarding the lymphocyte Fcγ-R. However, these receptors are not necessarily identical, and Colombatti, Heumann & Moretta (1981) found PMN Fcγ-R to be resistant to very high concentrations of pronase, in contrast to lymphocyte Fcγ-R. The mechanism by which the Fcγ-R levels are reduced by doxycycline is not clear, but the divalent cation chelating effect of the tetracyclines (Gnarpe & Leslie, 1974) may be of importance as the addition of 4 mM Mg^{2+} reversed the Fcγ-R decrease caused by 50 μg/ml of doxycycline.

The decreased percentage of Fcγ-R bearing cells after doxycycline incubation combined with PUVA irradiation (Table 3) suggests photo damage to the cell membrane as one possible mechanism. This combined treatment reduced the levels of Fcγ-R positive granulocytes to about one-half of the pre-treatment levels, whereas irradiation alone had no significant effect.

Tetracyclines are potent photosensitizers and patients taking tetracyclines often develop skin lesions when exposed to sunshine (Harber, Kochevar & Shalita, 1982). Which subcellular structures are damaged during irradiation of granulocytes in the presence of doxycycline is not known, but both the photodamage to the Fcγ-R receptor and the photodamage to granulocyte locomotion and phagocytosis (Sandberg *et al.*, 1984) can be explained by deterioration of the plasma membrane. Tetracyclines have also been shown to influence lymphocyte functions. Banck & Forsgren (1979) found low concentrations of doxycycline *in vitro* to have an inhibiting effect on the mitogenic responses of T and B lymphocytes, as well as on *in vitro* antibody production, and protein synthesis in unstimulated lymphocytes. Mice treated with tetracycline have been shown to have a reduced capacity to mount delayed-type hypersensitivity to sheep E (Thong & Ferrante, 1980). The present study has not, however, demonstrated any significant difference in the proportion of Fcγ-R, C3b-R or E-R bearing lymphocytes during doxycycline treatment. Neither had our patients low proportions of Fcγ-R bearing lymphocytes, as was found by Valmin, Halberg & Hedström (1982) in patients with severe staphylococcal furunculosis. The essentially unchanged percentage of E-RFC during doxycycline treatment does not support the claim of Pudifin *et al.* (1978) that tetracycline treatment causes an increase in lymphocytes bearing both E-R and surface immunoglobulins.

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REFERENCES

BANCK, G. & FORSGREN, A. (1979) Antibiotics and supression of lymphocyte function *in vitro*. *Antimicrob. Agents Chemother.* **16,** 554.

BELSHEIM, J., GNARPE, H. & LØFBERG, J. (1979) Granulocyte function during prophylaxis with doxycycline. *Scand. J. Infect. Dis.* **11,** 287.

COLOMBATTI, M., HEUMANN, D. & MORETTA, L. (1981) Distribution and properties of Fc receptors for IgG on different leukocyte populations in man. *Clin. exp. Immunol.* **46,** 453.

FORSGREN, A. & GNARPE, H. (1973) Tetracycline interference with the bactericidal effect of serum, *Nature*, **244,** 82.

FORSGREN, A., SCHMELING, D. & QUIE, P.G. (1974) Effect of tetracycline on the phagocytic function of human leukocytes. *J. Infect. Dis.* **130,** 412.

GLETTE, J., HALSTENSEN, A., SANDBERG, S., HOPEN, G., HELLUM, K.B. & SOLBERG, C.O. (1982) Effect of doxycycline and PUVA light on human polymorphonuclear leukocyte function. In *The Influence of Antibiotics on the Host-Parasite Relationship* (ed. by H. U. Eickenberg, H. Hahn & W. Opferkuch) p. 33, Springer-Verlag, Berlin, FRG.

GNARPE, H. & LESLIE, D. (1974) Tetracyclines and host defence mechanisms. Doxycycline interference with phagocytosis of *Escherichia coli*. *Microbios.* **10A,** 127.

HALLBERG, T., GURNER, B.W. & COOMBS, R.R.A. (1973) Opsonic adherence of sensitized ox red cells to human lymphocytes as measured by rosette formation. *Int. Archs. Allergy appl. Immunol.* **44,** 500.

HARBER, L.C., KOCHEVAR, I.E. & SHALITA, A.R. (1982) Mechanisms of photosensitization to drugs in humans. In *The Science of Photobiology* (ed. by J. D. Regan & J. A. Parrish) p. 323, Plenum, New York, USA.

KLEMPNER, M.S. & GALLIN, J.I. (1978) Separation and functional characterization of human neutrophil subpopulations. *Blood*, **51,** 659.

MATRE, R. & TØNDER, O. (1976) Complement receptors in human renal glomeruli. *Scand. J. Immunol.* **5,** 437.

NÆSS, A. & NYLAND, H. (1978) Multiple sclersosis. T lymphocytes in cerebrospinal fluid and blood. *Eur. Neurol.* **17,** 61.

PUDIFIN, D.J., DUURSMA, J., FORMO, R. & BRAIN, P. (1978) 'Double marker' lymphocytes in patients receiving tetracycline. *Clin. exp. Immunol.* **34,** 143.

REBORA, A., DALLEGRI, F. & PATRONE, F. (1979) Neutrophil functions in acne conglobata. *Dermatologica*, **159,** 217.

SANDBERG, S., GLETTE, J., HOPEN, G. & SOLBERG, C.O. (1984) Doxycycline induced photodamage to human neutrophils and tryptophan. *Photochem. Photobiol.* **39,** 43.

SMITH, R., KERMAN, R., EZDINLI, E. & STEFANI, S. (1975) A modified assay for the detection of human adult active rosette forming lymphocytes. *J. Immunol. Methods*, **8,** 175.

TALSTAD, I. (1981) Simultaneous separation of monocytes, lymphocytes and polymorphonuclear cells from the peripheral blood. International Society of Haematology, European and African Division. 6th Meeting, Athens.

THONG, Y.H. & FERRANTE, A. (1980) Effect of tetracycline treatment on immunological responses in mice. *Clin. exp. Immunol.* **39,** 728.

VALMIN, K., HALLBERG, T. & HEDSTRØM, S.A. (1982) Recurrent staphylococcal furunculosis: Lymphocyte subsets and plasma immunoglobulins. *Scand. J. Infect. Dis.* **14,** 152.

WEENING, R.S., ROOS, D. & LOOS, J.A. (1974) Oxygen consumption of phagocytizing cells in human leukocyte and granulocytes preparations: a comparative study. *J. Lab. Clin. Med.* **83,** 570.

WYBRAN, J. & FUDENBERG, H.H. (1973) Thymus derived rosette-forming cells. *New Engl. J. Med.* **288,** 1072.