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# NOTES

## Quinolones Affect Thymidine Incorporation into the DNA of Human Lymphocytes

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**The incorporation of [$^3$H]thymidine into DNA was increased in phytohemagglutinin-stimulated human lymphocytes exposed to four of the new quinolone derivatives (ciprofloxacin, norfloxacin, ofloxacin, and A 56620) at concentrations achievable in clinical situations. However, proliferation of phytohemagglutinin-stimulated lymphocytes was not influenced by ciprofloxacin at concentrations of 0.5 to 10 μg/ml.**

In previous studies from our laboratory, we reported the inhibitory effects of various antibiotics on the mitogenic response of human lymphocytes (3, 7, 9). Some of these antibiotics (e.g., tetracyclines, rifampin, and fusidic acid) have also been shown to impair the immune response in vivo (4–6, 10, 12–14). Despite the inhibitory effects of some of the antibiotics, none of those tested so far have been reported to stimulate human lymphocytes.

Ciprofloxacin, norfloxacin, and ofloxacin are new quinolone derivatives, structurally related to nalidixic acid but far more potent; they are effective against a wider range of bacteria—even against such bacteria as *Pseudomonas aeruginosa* and gram-positive cocci, which are considered to be resistant to nalidixic acid. In a previous study (A. Forsgren and P. I. Bergkvist, Eur. J. Clin. Microbiol., in press), we found no direct effect of quinolones on the function of human granulocytes. The aim of the present study was to examine the in vitro function of human T and B lymphocytes in the presence of quinolone antibiotics.

Fresh solutions of the following preservative-free antibiotics were used: ciprofloxacin (lot no. 907 337; Bayer, Wuppertal, Federal Republic of Germany), norfloxacin (lot no. 021 8020; Astra, Södertälje, Sweden), ofloxacin (lot no. 10-01; Hoechst, Frankfurt, Federal Republic of Germany), A 56619 and A 56620 (lot no. 64-294 AL0 and 66-298 AL, respectively; Abbott Laboratories, North Chicago, Ill.), and nalidixic acid (lot no. DYK 1454; Sterling Drug, Inc., New York, N.Y.).

Heparinized blood from healthy laboratory personnel was used in all experiments. Lymphocytes were harvested by centrifugation on a step gradient of mixed Isopaque (1 part) and Macrodex (2 parts), followed by separation on Ficoll-Isopaque (Lymphoprep; Pharmacia, Uppsala, Sweden). The cells (more than 99% were mononuclear) were washed twice in RPMI 1640 (Gibco Bio-Cult, Ltd., Edinburgh, Scotland).

Phytohemagglutinin (PHA) (Wellcome Research Laboratories, Beckenham, England) was used at a final concentration of 1 μg/ml. The Cowan 1 strain of *Staphylococcus aureus* and a clinical isolate of *Branhamella catarrhalis* (*B. catarrhalis* 5) were used as B-cell mitogens (2, 11). *S. aureus* Cowan 1 was grown overnight in CCY broth (1), and *B. catarrhalis* was grown on Paper Disc Method agar (Biodisk, Solna, Sweden). After being harvested, the bacteria were killed by suspension in 0.5% formaldehyde for 3 h at room temperature, followed by heat treatment at 80°C for 3 min. After being washed in phosphate-buffered saline, the bacteria were suspended in RPMI 1640 and stored at −20°C. The killed bacteria were added to lymphocyte cultures to a final concentration of 0.1%.

Triplicate lymphocyte samples were cultured in microtiter plates containing $3 \times 10^5$ lymphocytes per culture in 200 μl of RPMI 1640 supplemented with 1% AB serum and 12 μg of gentamicin per ml. The effect of quinolone antibiotics on human lymphocytes was identical in gentamicin-free medium. Quinolone antibiotics and mitogens were added at the beginning of the experiments, and the cultures were incubated for 3 days in 5% $CO_2$ at 37°C. The pH of the culture medium was unchanged at the end of the experiment.

A 2-μCi portion of [$^3$H]thymidine (NET-027Z; specific activity, 78 Ci/mmol; New England Nuclear Corp., Boston,

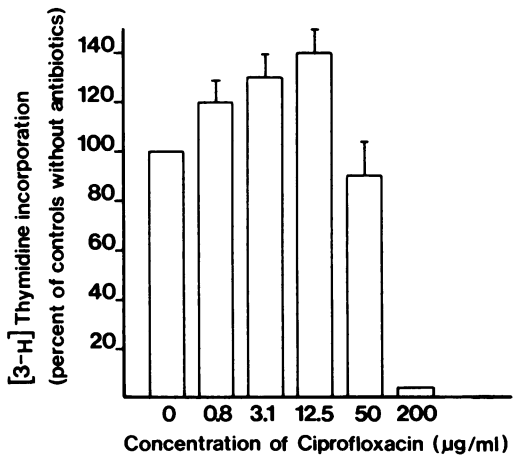


FIG. 1. Response ([$^3$H]thymidine incorporation) of PHA-stimulated human lymphocytes in the presence of ciprofloxacin. The results (mean ± standard deviation), which are given as percentages of incorporation in controls without antibiotics, represent experiments with lymphocytes from seven volunteers. The increase in concentrations (0.8 to 12.5 μg/ml) is statistically significant ($P < 0.01$).

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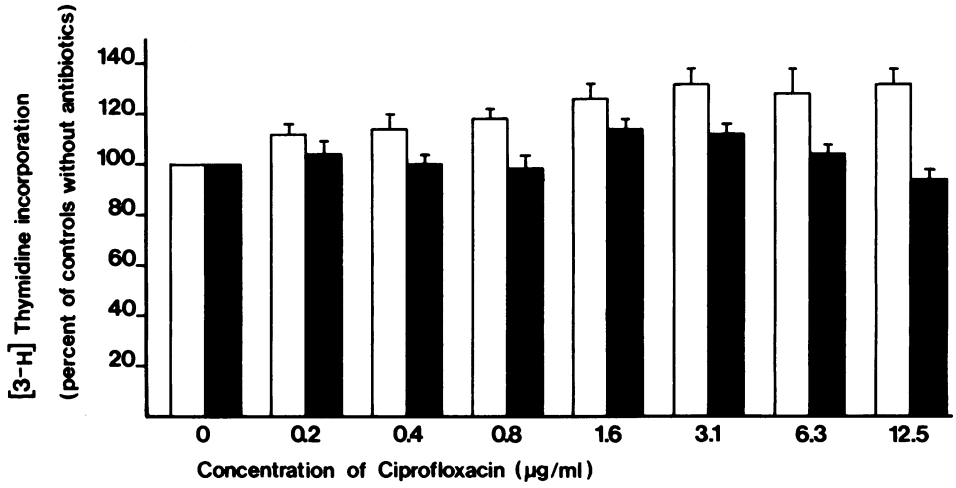


FIG. 2. Response ([$^3$H]thymidine incorporation) of *S. aureus* Cowan 1- and *B. catarrhalis*-stimulated human lymphocytes in the presence of ciprofloxacin. The results (mean ± standard deviation) are given as percentages of incorporation in controls without antibiotics. The increased response to *B. catarrhalis* (□) is significant ($P < 0.01$) at all concentrations. The response to *S. aureus* Cowan 1 (■) is increased ($P < 0.01$) at ciprofloxacin concentrations of 1.6 and 3.1 μg/ml.

Mass.) in RPMI 1640 was added 20 h before the cultures were harvested onto glass fiber filters with distilled water by use of a Skatron harvesting machine (Skatron A/S; Liberbyen, Norway). In some experiments, the filters were carefully washed with 10% trichloroacetic acid after the cultures were harvested. The filters were dried and transferred to scintillation vials containing 2 ml of Insta-Fluor solution (Packard Instrument Co., Inc., Rockville, Md.). The radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, and data were calculated from the median of triplicate cultures and expressed as percentages of antibiotic-free controls. Wilcoxon's matched-pair test was used to check the statistical significance of results.

Initially, the effect of ciprofloxacin on proliferation and [$^3$H]thymidine uptake in stimulated T and B lymphocytes was measured. When cells were counted in an automatic cell counter after 3 days of stimulation with PHA, they were two to three times as numerous as on day 1 of culturing. In many experiments, no effect on cell proliferation was detected for ciprofloxacin at concentrations of 0.5 to 10 μg/ml. However, ciprofloxacin at concentrations achievable in serum in clinical situations (0.8 to 12.5 μg/ml) significantly increased [$^3$H]thymidine incorporation in PHA-stimulated human lymphocytes (Fig. 1). The effect of ciprofloxacin on human lymphocytes stimulated with the B-cell mitogens *B. catarrhalis* and *S. aureus* Cowan 1 is shown in Fig. 2. The response to *B. catarrhalis* was increased by ciprofloxacin ($P < 0.01$) at all concentrations, whereas the response to Cowan 1 was increased by ciprofloxacin at concentrations of 1.6 and 3.2 μg/ml. The actual incorporation of [$^3$H]thymidine in the absence of ciprofloxacin was 60,000 to 100,000 cpm, 25,000 to 30,000 cpm, and 15,000 to 20,000 cpm for lymphocytes stimulated with PHA, *B. catarrhalis*, and Cowan 1, respectively. This demonstrated the relatively stronger effect of ciprofloxacin on lymphocyte cultures with a higher [$^3$H]thymidine incorporation level.

The effect of five different new quinolones and nalidixic acid on [$^3$H]thymidine incorporation in PHA-stimulated human lymphocytes (T lymphocytes) is shown in Table 1. At some concentrations, four of these five new quinolones significantly increased [$^3$H]thymidine uptake, ciprofloxacin being most active and norfloxacin and ofloxacin showing a slightly lower activity. The quinolone derivative A 56620 was less active than norfloxacin or ofloxacin, and no significant effect was seen for A 56619. At concentrations up to 200 μg/ml, nalidixic acid did not affect [$^3$H]thymidine uptake, but at a nalidixic acid concentration of 400 μg/ml, [$^3$H]thymidine incorporation was reduced to approximately 25%.

The results demonstrated in Fig. 1 and 2 and Table 1 are based on lymphocyte cultures harvested onto glass fiber filters with distilled water. Careful washing with trichloroacetic acid, however, did not influence the results. This shows that [$^3$H]thymidine was incorporated into DNA.

TABLE 1. [$^3$H]thymidine incorporation in PHA-stimulated human lymphocytes in the presence of different quinolone derivatives

| Quinolone derivative | [$^3$H]thymidine incorporation[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 50 | 25 | 12.5 | 6.25 | 3.12 | 1.56 | 0.78 | 0.39 | 0.20 |
| Ciprofloxacin | 122 ± 9[b] | 150 ± 11[b] | 143 ± 8[b] | 141 ± 10[b] | 121 ± 11[c] | 117 ± 5[b] | 106 ± 6 | 107 ± 7 | 103 ± 7 |
| Norfloxacin | 74 ± 43 | 140 ± 16[b] | 133 ± 17[c] | 129 ± 13[c] | 114 ± 8[b] | 112 ± 5[c] | 95 ± 7 | 93 ± 11 | 98 ± 8 |
| Ofloxacin | 152 ± 13[b] | 147 ± 10[b] | 134 ± 9[b] | 123 ± 8[c] | 118 ± 8[c] | 106 ± 8 | 104 ± 6 | 99 ± 8 | 98 ± 4 |
| A 56619 | 89 ± 15 | 108 ± 11 | 114 ± 8 | 109 ± 10 | 101 ± 10 | 106 ± 8 | 101 ± 9 | 104 ± 9 | 98 ± 8 |
| A 56620 | 140 ± 15[b] | 134 ± 14[c] | 123 ± 10[c] | 114 ± 10[c] | 110 ± 8[c] | 100 ± 8 | 101 ± 8 | 96 ± 8 | 103 ± 7 |
| Nalidixic acid | 89 ± 8 | 88 ± 8 | 89 ± 7 | 93 ± 6 | 97 ± 6 | 89 ± 6 | NT[d] | NT | NT |

[a] Expressed as a percentage of [$^3$H]thymidine incorporation in controls ± standard error of the mean.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] NT, Not tested.

The first lymphocyte-activating substance described (PHA) later turned out to be selective for T lymphocytes, and the response has usually been assessed by measuring the uptake of radioactive thymidine into DNA. We previously described *S. aureus* Cowan 1 and *B. catarrhalis* as being effective mitogens for B lymphocytes (3, 8). Many different antibiotics have no effect, and a few inhibit the mitogenic response of human T and B lymphocytes (3, 7, 9). The finding that lymphocytes show increased incorporation of [$^3$H]thymidine in the presence of quinolones is therefore remarkable and unique. The results are even more interesting because, with some of the new quinolones, the effect is even apparent at clinically achievable concentrations. The stimulatory effect on [$^3$H]thymidine uptake in PHA-stimulated cells was most pronounced in the presence of ciprofloxacin, which has the strongest antibacterial effect (8). Therefore, the capacity of the new quinolones to induce [$^3$H]thymidine incorporation into DNA in mitogen-stimulated lymphocytes seems to be related to their reported antibacterial effect.

The stimulatory effect of quinolones on [$^3$H]thymidine uptake suggests an enhancement of immune function. However, enhanced DNA synthesis in the absence of enhanced proliferation could indicate that increased [$^3$H]thymidine uptake is a sign of changed DNA synthesis caused by the new quinolones at clinically achievable concentrations. The reason for and consequences of the increased [$^3$H]thymidine incorporation into DNA in the presence of the new quinolones will be evaluated by us in additional studies.

## LITERATURE CITED


1. **Arvidson, S., T. Holme, and T. Wadström.** 1970. Formation of bacteriolytic enzymes in batch and continuous culture of *Staphylococcus aureus*. J. Bacteriol. **104**:227–233.
2. **Banck, G., and A. Forsgren.** 1978. Many bacterial species are mitogenic for human blood B lymphocytes. Scand. J. Immunol. **8**:347–354.
3. **Banck, G., and A. Forsgren.** 1979. Antibiotics and suppression of lymphocyte function in vitro. Antimicrob. Agents Chemother. **16**:554–560.
4. **Bellahséne, A., and A. Forsgren.** 1980. Effect of rifampin on the immune response in mice. Infect. Immun. **27**:15–20.
5. **Bellahséne, A., and A. Forsgren.** 1980. Effect of fusidic acid on the immune response in mice. Infect. Immun. **29**:873–878.
6. **Bellahsene, A., and A. Forsgren.** 1985. Effect of doxycycline on immune response in mice. Infect. Immun. **48**:556–559.
7. **Forsgren, A.** 1981. The immunosuppressive effect of cephalosporins and cephalosporin-gentamicin combinations. J. Antimicrob. Chemother. **8**(Suppl. B):183–186.
8. **Forsgren, A.** 1985. Comparative in vitro activity of three new quinolone antibiotics against recent clinical isolates. Scand. J. Infect. Dis. **17**:91–94.
9. **Forsgren, A., and G. Banck.** 1978. Influence of antibiotics on lymphocyte function in vitro. Infection **6**(Suppl.):91–97.
10. **Forsgren, A., G. Banck, H. Beckman, and A. Bellahséne.** 1980. Antibiotic host defence interactions in vitro and in vivo. Scand. J. Infect. Dis. Suppl. **24**:195–203.
11. **Forsgren, A., A. Svedjelund, and H. Wigzell.** 1976. Lymphocyte stimulation by protein A of *Staphylococcus aureus*. Eur. J. Immunol. **6**:207–213.
12. **Rijkers, G. T., R. Van Oosterom, and W. B. Van Muiswinkel.** 1981. The immune system of cyprinid fish. Oxytetracycline and the regulation of humoral immunity in carp (*Cyprinus carpio*). Vet. Immunol. Immunopathol. **2**:281–284.
13. **Thong, Y. H., and A. Ferrante.** 1980. Effects of tetracycline treatment on immunological responses in mice. Clin. Exp. Immunol. **39**:728–732.
14. **Van den Bogert, C., and A. M. Kroon.** 1982. Effects of oxytetracycline on in vivo proliferation and differentiation of erythoid and lymphoid cells in rat. Clin. Exp. Immunol. **50**:327–335.