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Effect of Antibiotics on Chemotaxis of Human Leukocytes

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The effect of 20 different antibiotics on chemotaxis by human neutrophils was studied. Human leukocytes incubated with chloramphenicol, rifampin, sodium fusidate, and tetracyclines in vitro showed markedly depressed migration. The mechanisms by which these antibiotics affect leukotaxis are discussed.

Leukotaxis may be defined as the directional migration of leukocytes toward a chemotactic stimulus and is believed to represent the means by which a massive influx of polymorphonuclear leukocytes occurs in an area of inflammation (30). The attraction of polymorphonuclear leukocytes is brought about by a series of effector substances involving complement components (27), bacterial culture filtrates (14, 28), extracts from the leukocytes themselves (6), and tissues infected with virus (25, 31).

The possible role of drugs on the inflammatory system has received increasing attention. Martin et al. (18, 19) recently reported an inhibitory effect of tetracycline on leukocyte migration, which was discovered during a serial study of leukotaxis using human leukocytes and sera. The purpose of the present study was to extend earlier studies on the effects of antibiotics other than tetracyclines on the chemotaxis of human neutrophils.

MATERIALS AND METHODS

Antibiotics. A wide range of antibiotics was tested: sulfametoxazol (Kabi AB, Sweden, batch 10708-52), trimethoprim (Syraprim, Burroughs Wellcome Co., batch 55034), nalidixic acid (Winthrop Laboratories, batch K1 1770), nitrofurantoin (Pharmacia Fine Chemicals, Sweden, batch T 8533), ampicillin (aminobenzylpenicillin, Astra AB, Sweden, batch 175), benzylpenicillin (Astra AB, Sweden, batch 61062-01), carboxybenzylpenicillin sodium (Fugacillin, Astra AB, Sweden, batch 50428-02), cefazolin sodium (Lilly Research Centre Ltd., batch 7 KK43), cephalothin sodium (Lilly Research Centre Ltd., batch 215-DJ2-1), doxycycline hyclate (Pfizer, batch 312-58709), lymecycline (Carlo Erba, Italy, batch 6 D 5154 9764), tetracycline chloride (Dumex, batch 308296), gentamicin sulfate (San Bolagen AB, Sweden, batch 6694), kanamycin (Ferrosan, Sweden, batch 03205 Z9), chloramphenicol succinate (Carlo Erba, Italy, batch 730767), erythromycin (Upjohn, batch 2324 W), clindamycin hyclate (Cleocin, Upjohn, batch U-21251 FIEK 55), rifampin (Ferrosan, Sweden, batch 4422:T) and sodium fusidate (Leo Pharmaceutical Products, Denmark, batch 22194). Fresh stock solutions were prepared for all experiments, both in water, for use in the agarose medium, and in tissue culture medium 199, for preincubation with leukocytes.

Leukocytes. Heparinized venous blood was drawn from healthy volunteers. Erythrocytes were allowed to sediment by gravity at room temperature for a period of about 1 h. The leukocyte-rich plasma was then removed, and the concentration of polymorphonuclear leukocytes was adjusted to 10^7/ml of plasma.

Chemotaxis studies. Leukocyte chemotaxis was studied by a modification of the method described by Nelson et al. (21). Briefly, agarose plates were prepared containing 0.5 ml of 10× tissue culture medium 199 (Flow Laboratories), 0.15 mmol of HEPES (*N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid buffer (Schwarz/Mann, Div. of Becton Dickinson & Co., Orangeburg, N. Y.), 0.5 ml of 5% agarose (Litex, Glostrup, Denmark), and the desired concentration of the antibiotics to be tested. The pH of the medium was adjusted to 7.4 before addition of the agarose. Five milliliters of the agarose solution was then transferred to a tissue culture dish (60 by 15 mm; 3002, Falcon, Oxnard, Calif.) and allowed to harden. Wells with a diameter of 3 mm and spaced 3 mm apart were cut. Each plate contained five series of three wells.

In most experiments, 0.5 ml of a plasma suspension containing 10^7 polymorphonuclear leukocytes per ml was preincubated with 0.5 ml of the various antibiotic solutions in 1× tissue culture medium 199 (National Bacteriological Laboratory, Stockholm, Sweden) for 30 min in a shaking water bath at 37°C. After centrifugation for 10 min at 200 × *g*, the cell pellet was resuspended in a small portion of the supernatant to give a final concentration of 10^8 polymorphonuclear leukocytes per ml.

Cell suspensions preincubated with antibiotics were added to the middle wells in three of the five well sets in the agarose plates with the corresponding concentration of antibiotics. The remaining two middle wells were filled with 10 μl of plasma, containing 10^8 polymorphonuclear leukocytes per ml, which had been kept free of any antibiotic contact. The five inner wells were all filled with 10 μl of a chemotactically active *Escherichia coli* culture filtrate (28), and the outer wells were filled with 10 μl

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of 1× tissue culture medium. Incubation was carried out for a period of 3 h at 37°C in a humidified atmosphere containing 5% CO_2 in air. The plates were then fixed and stained, and the chemotaxis was quantitated by measurement of a greatly enlarged projection of the migration patterns; i.e., the number of millimeters from the wells was measured.

Results were calculated as percentages of control values that were obtained using cells preincubated with tissue culture medium without antibiotics and tested for chemotactic capacity in plates lacking antibiotic content but otherwise identical to the test plates. Plate variation was checked by comparing migration zones for the cells that had not been preincubated with an antibiotic. The reproducibility of the method was determined: 100 ± 7.78 (standard deviation); 0.9431 (standard error).

RESULTS

Rifampin. Figure 1 shows the result of one typical experiment out of five. When normal human polymorphonuclear leukocytes were incubated with rifampin for 30 min and then placed in an agarose gel not containing rifampin, the directional migration (leukotaxis) toward an *E. coli* filtrate was significantly inhibited and, at a rifampin concentration of 25 μg per ml, the chemotactic movement after 3 h of incubation was only 50% of that obtained with cells that had been preincubated with tissue culture medium without antibiotics. When rifampin was included in the agarose gel used for the chemotaxis experiment in the same concentration as in the medium used for preincubation, an even more pronounced effect was seen and, at a rifampin concentration of 2.5 μg per ml, the chemotaxis movement was 50% of the control value obtained with cells that had been preincubated in tissue culture medium and tested in a gel without antibiotics.

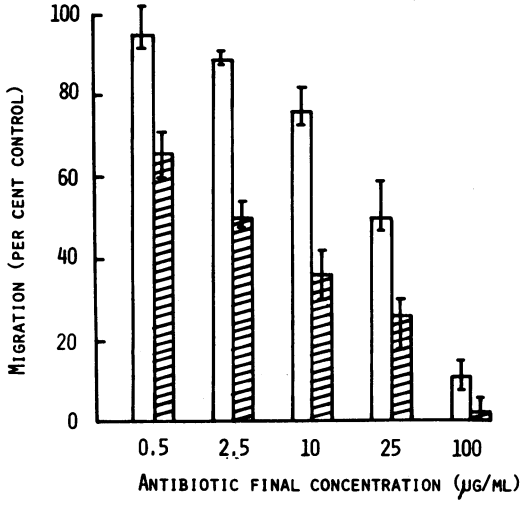

FIG. 1. *Chemotaxis of normal human polymorphonuclear leukocytes preincubated with rifampin and then concentrated and placed in agarose gel (mean and range of three determinations). Open bars indicate the chemotaxis in gels that were free of antibiotics, and the shaded bars represent the chemotaxis of neutrophils in antibiotic-containing gels. Symbols: (□) cells preincubated with rifampin, (▨) cells preincubated with and placed in gels containing rifampin.*

Tetracyclines. Figure 2 demonstrates the effect of three different tetracyclines on chemotaxis. The results signify a series of experiments using polymorphonuclear leukocytes from different healthy volunteers. The neutrophils were preincubated with the drugs and then added to chemotaxis plates containing the corresponding antibiotic concentration. At therapeutically attainable concentrations, the effect of tetracyclines was slight. However, at higher concentrations (25 to 100 μg/ml), the effect was striking, especially with doxycycline, for which (at a concentration of 100 μg per ml) the neutrophils did not move on the chemotaxis plate. Control cells not preincubated with antibiotics but placed in agarose plates containing antibiotics showed the same degree of migration as those incubated in antibiotic-free plates. This shows that the preincubation with tetracyclines was necessary for an effect on chemotaxis and also that the effect detected was an effect on the cells and not merely an effect of tetracyclines on the agarose plate.

Chloramphenicol and fusidic acid. Figure 3 shows a representative experiment with chlor-

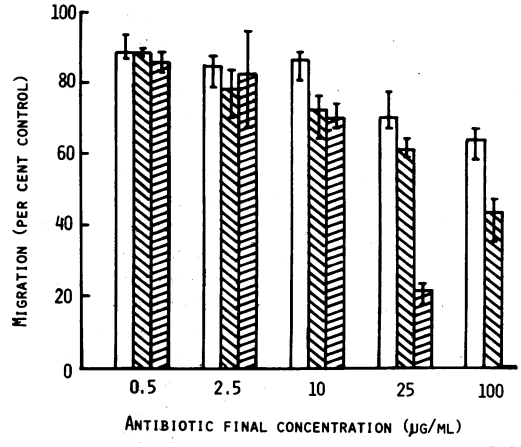

FIG. 2. *Chemotaxis of normal human neutrophils preincubated with tetracyclines for 30 min and then concentrated and tested in agarose gels containing the same concentration of the corresponding antibiotic (mean and range of three determinations). Symbols: (□) tetracycline chloride, (▧) lymecycline, (▨) doxycycline.*

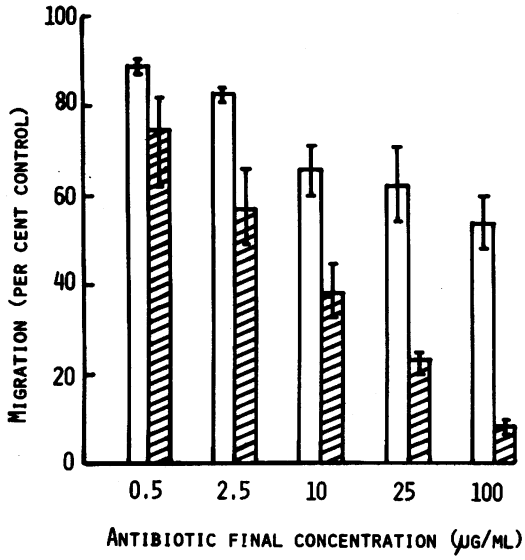

FIG. 3. *Effects of chloramphenicol and fusidic acid on chemotaxis of normal human neutrophils that were preincubated with different concentrations of the drugs and then tested in plates containing the corresponding antibiotic concentrations (mean and range of three determinations). Symbols: (□) chloramphenicol, (▨) fusidic acid.*

amphenicol and sodium fusidate similar to that described above for tetracyclines. The figure reflects a very strong depressive effect of sodium fusidate on neutrophil chemotaxis and, at a level of 10 μg per ml, the chemotactic migration was only 38% of control values. The corresponding figure for chloramphenicol was 66%. At higher concentrations, a more marked effect was shown with both drugs and, at 25 and 100 μg per ml, the observed chemotaxis values were 62 and 54, respectively, 23 and 8% of control values for chloramphenicol and sodium fusidate.

Other antibiotics. For the remaining antibiotics tested, chemotaxis values were generally within 15% of the control values, a range that we considered nonindicative of any significant depressive effect upon chemotactic migration. Exceptions included a slightly depressive effect by clindamycin, erythromycin, and nitrofurantoin in the highest concentration (100 μg/ml) tested. For penicillins and cephalosporins, no appreciable effect on chemotaxis could be detected. Nor was there any effect of sulfa, Syraprim, gentamicin, kanamycin, colistin, or nalidixic acid.

DISCUSSION

Chemotaxis and phagocytosis are both important steps in the major defence mechanism against pyogenic infections. Very few studies on the effect of antibiotics on chemotaxis of polymorphonuclear leukocytes have been reported (18, 19).

Rifampin is of special interest, due to its capacity to penetrate leukocytes and destroy intracellular microorganisms in vitro and probably in vivo (17). Hoeprich et al. (13) previously demonstrated that rifampin did not interfere with phagocytosis and intracellular killing. However, Urbanitz et al. (26), when comparing the function of monocytes from 15 patients with tuberculosis under treatment with rifampin, detected a significantly impaired phagocytosis of immunoglobulin G-coated erythrocytes. Several authors have reported on the influence of rifampin upon immunological reactions (11, 23, 24).

In the present study, a significant effect of rifampin on the chemotaxis of human polymorphonuclear leukocytes was obtained. The effect was most pronounced when human cells, which had been preincubated with rifampin for 30 min, were applied in a gel plate containing the same concentration of the drug, but cells that were preincubated with rifampin and then applied to a gel without antibiotics were also significantly inhibited. Rifampin has been shown to inhibit deoxyribonucleic acid-dependent ribonucleic acid (RNA) polymerase activity (12) in susceptible bacterial cells. However, the basis for the effect of rifampin on chemotaxis, as reported in this paper, is unknown.

Many reports are available concerning the effects of tetracycline on the phagocytic system (1, 13, 20). Most recently, in a study by Forsgren et al. (8), it was demonstrated that polymorphonuclear leukocytes incubated in vitro with tetracycline hydrochloride or doxycycline showed a decreased capacity to phagocytize yeast and bacteria. Furthermore, leukocytes harvested from healthy volunteers after ingestion of tetracycline also demonstrated decreased phagocytic capacity. By scanning electron microscopy, Gnarpe and Leslie (10) could detect distinct surface alterations in human leukocytes; they noted that bacteria adhered to, but were not ingested by, the leukocytes when doxycycline was present. Gnarpe and Leslie (10) attributed the inhibitory effect of tetracycline to antibiotic complex binding of membrane-bound divalent cations. Those ions are necessary for both the phagocytic and chemotactic function of polymorphonuclear leukocytes (3, 9, 32).

Martin et al. (18, 19) reported on inhibiting effect of tetracyclines on leukotaxis. The present study confirms their findings. The incubation of human blood cells with tetracyclines

results in considerably higher concentrations intracellularly than in the surrounding medium (22). The differences in ability to impair chemotaxis between doxycycline in high concentrations and other tetracyclines might be due to the fact that doxycycline is more lipid soluble (5) than the other tetracyclines.

Earlier studies on the effect of chloramphenicol on phagocytic cells have failed to show any effect at therapeutic concentrations of the drug. Lehrer (16), Downey and Pisana (7), and Odegaard and Lamvik (33) could only detect an effect on phagocytosis by chloramphenicol at very high concentrations of the drug. Kernbaum (15), however, reports no depression of phagocytosis by human neutrophils with high concentrations (1 mg/ml) of chloramphenicol. However, it has been suggested that chloramphenicol inhibits antibody production by interfering with some early phase of the response, and it was hypothesized that the interference involves messenger RNA (2). The basis for the effect of chloramphenicol on human neutrophil leukocytes, as described in this paper, has not been further studied, but it seems to be most pronounced for concentrations of the drug that are above therapeutic levels.

Fusidic acid is, like chloramphenicol, an inhibitor of protein synthesis. Fusidic acid inhibits the transfer of transfer-RNA-bound amino acids to ribosomes. In the chemotaxis system, used by us, sodium fusidate had a pronounced effect on chemotaxis, even in therapeutic levels. The basis for this effect warrants further studies.

Carruthers (4) reported that drugs which inhibit RNA and protein synthesis, such as puromycin at 10^3 M and actinomycin D at 10 μg per ml, had a slight inhibitory effect on random migration of neutrophils but a complete inhibitory effect on directional migration. It is of great interest that all antibiotics tested in this investigation with a strong inhibitory effect on human leukotaxis have an inhibitory effect on either transcription or protein synthesis. Thus, it is possible that a protein(s) necessary for the chemotactic response is synthesized on contact of the cell with chemotactic factors and that this synthesis can be blocked at the level of transcription or later, thus blocking the response of the cell.

Clindamycin and erythromycin, which also inhibit protein synthesis, seemingly had no effect on the chemotaxis of polymorphonuclear leukocytes, except at higher concentrations, where a decreasing effect was seen. As might be expected, no effect was found when the beta-lactam antibiotics benzylpenicillin, ampicillin, Fugacillin, cefazolin, and cephalothin were tested. Penicillin G was tested in phagocytosis systems by Downey and Pisana (7) and Kernbaum (15) without any detectable effect. Gentamycin and trimethoprim have been reported by Kernbaum (15) to have no detectable effect on phagocytosis. Nor could we detect any effect on chemotaxis. Sulfonamides have been reported to inhibit myeloperoxidase-mediated bactericidal reactions of polymorphonuclear leukocytes. However, there have been no reports on the effect of sulfonamides on phagocytic uptake and chemotaxis. This study reveals no effect of sulfonamides on chemotaxis. Nor could any effect on chemotaxis be detected for nalidixic acid and nitrofurantoin.

ACKNOWLEDGMENTS

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