Comparative Effects of Moxalactam and Gentamicin on Human Polymorphonuclear Leukocyte Functions

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The in vitro effects of moxalactam and gentamicin on chemotactic, phagocytic, and microbicidal activities of human polymorphonuclear leukocytes have been studied. Incubation of cells with moxalactam (25 to 400 µg/ml) resulted in a 10 to 15% inhibition of leukocyte chemotaxis ($P \leq 0.10$). Incubation of cells with gentamicin (5 to 40 µg/ml) inhibited chemotaxis more than 50% ($P \leq 0.50$). Neither moxalactam nor gentamicin had any effect on phagocytic or microbicidal capacities of leukocytes.

Several antimicrobial agents purportedly interfere with the functions of human polymorphonuclear (PMN) cells (1, 3). Erythromycin, tetracycline, rifampin, aminoglycosides, and chloramphenicol inhibit the chemotactic activity of granulocytes in vitro (4, 8, 12, 14). Most beta-lactam drugs do not affect leukocyte functions but cefoxitin at therapeutic levels has recently been shown to interfere with the chemotaxis of PMN cells in vitro (13).

Moxalactam, a relatively new beta-lactam active in vitro against a broad spectrum of facultative and anaerobic bacteria, can frequently replace aminoglycosides such as gentamicin. It is important therefore to know how these agents affect leukocyte functions.

The purpose of our work has been to compare effects of moxalactam and gentamicin on migration, phagocytosis, and microbicidal capacity of human PMN leukocytes.

MATERIALS AND METHODS

Antibiotics. Moxalactam (provided by Eli Lilly & Co.) was suspended in 0.1 M phosphate buffer (pH 7), and gentamicin (provided by Schering Corp.) was suspended in distilled water. In all experiments, dilutions from each antibiotic were prepared in Hanks solution.

Leukocytes. Heparinized venous blood was taken from healthy volunteers and was allowed to sediment with 6% dextran T70 at room temperature. Leukocytes were washed twice, and erythrocytes were lysed with hypotonic shock. PMN cells were suspended in Hanks solution with 10% inactivated fetal calf serum to a concentration of 1×10^6 cells per ml. Cells were preincubated with different concentrations of moxalactam (final concentrations: 25, 50, 100, 200, and 400 µg/ml) and gentamicin (final concentrations: 5, 10, 20, and 40 µg/ml) in a shaking bath at 37°C for 30 min.

Chemotaxis. Chemotaxis of PMN cells was tested by

the agarose migration technique (15). Agarose solution was prepared on tissue culture medium 199 (Flow Laboratories) and complemented with 10% inactivated fetal calf serum and 1% glutamine at a final concentration of 0.75%. We then transferred 5 ml of the agarose solution to a tissue culture dish (60 by 15 mm; Corning 25010), and the solution was allowed to harden. Three series of three wells, 3 mm in diameter and spaced 2 mm apart, were cut in each plate. We added 10 µl of each cell suspension to the central wells of each plate. Interior wells were filled with 10 µl of Gey's solution for random migration. The three exterior wells of each plate were filled with 10 µl of zymosan-activated serum, which served as the chemotactic factor. Plates were incubated under a humidified atmosphere with 5% CO_2.

Cell migration was then read under the microscope ($\times 100$) with a microscale, using the difference between stimulated and random migration as effective migration.

Cells preincubated with various concentrations of moxalactam, gentamicin, and antibiotic-free cells were included in all chemotaxis experiments. The effect of antibiotics on cells was also measured in four separate experiments by the addition of 5 µl of preincubated cells or antibiotic-free cells to wells with 5 µl of different antibiotic concentrations. Control serum incubated with moxalactam (25 and 50 µg/ml) before incubation with zymosan was compared with a nonincubated serum for the effects of the drug on serum chemotactic factors.

Phagocytosis and microbicidal capacity. Phagocytosis and microbicidal capacity were measured against *Candida albicans* by the cytologic method of Lehrer (9) in four separate studies in which cells were preincubated with moxalactam (final concentration, 100 µg/ml) or with gentamicin (final concentration, 10 µg/ml), or were not previously incubated with either antibiotic. Results were expressed as a percentage of phagocytosis (number of cells with phagocyted organisms/total number of cells $\times 100$), phagocytic index (number of phagocyted organisms per cell), and microbicidal capacity (number of phagocyted and dead organisms/total number of phagocyted organisms $\times 100$).

RESULTS

Chemotaxis. Effective migration under agarose plates of PMN cells incubated with different concentrations of antibiotics and of control cells are summarized in Table 1. The effective migration of control cells was 113 ± 25 standard deviation and was not statistically different from that of cells incubated with 25 to 400 µg of moxalactam per ml ($P \leq 0.10$). The effective migration of PMN cells preincubated with 5 µg of gentamicin per ml was 51 ± 32, and similar values were obtained when gentamicin concentrations were 10, 20 and 40 µg/ml. These results are statistically significantly different from controls ($P \leq 0.05$, Student's t test). For better comparative purposes, migration is also expressed as a percentage in control cells. In all of the experiments conducted, the effective migration of leukocytes incubated with moxalactam was between 75 ± 21 and 90 ± 27% of that of control cells. When cells were incubated with gentamicin, their effective migration was 34 to 45% of that of controls (Table 1). Those results remained unchanged when antibiotics were added to the agarose wells. Chemotaxis was as effective in the presence of serum incubated with moxalactam as with nonincubated serum.

Phagocytosis and microbicidal capacity. The percentage of phagocytosis was 85 ± 8 and 84 ± 10% after cell exposure to moxalactam and gentamicin, respectively, and 78 ± 4% in the control group. The phagocytic index shows that each control preparation has a mean of 1.1 ± 0.2 candidas, each moxalactam preparation has a mean of 1.4 ± 0.2 candidas, and each gentamicin preparation has a mean of 1.5 ± 0.2 candidas; there is no statistically significant difference between any group ($P \leq 0.5$, Student's t test).

The numbers of candida lysed after ingestion were 41 ± 4 and 44 ± 4 in the moxalactam- and gentamicin-incubated cells and 48 ± 4 in the control cells. None of these three parameters was affected by the two antibiotics tested.

DISCUSSION

Various investigators have shown that the functional activity of human PMN leukocytes can be altered by certain antibiotics (2, 4, 10, 12). Tetracyclines, erythromycin, and rifampin (4, 11, 12, 14) reportedly interfere with chemotaxis of human PMN cells; results with chloramphenicol and aminoglycosides are contradictory (4, 6, 8, 12). Phagocytosis is inhibited by tetracycline or chloramphenicol (5, 7, 10), but is not modified by aminoglycosides or cephalosporins (13, 16, 17).

In our experiments with different concentrations of gentamicin, cell migration was inhibited

TABLE 1. Effect of moxalactam and gentamicin on chemotaxis of human PMN leukocytes

Antibiotics	Concn (µg/ml)	Migration[a]	
		Effective migration	Percentage of control
Control		113 ± 25	
Moxalactam	25	103 ± 31	90 ± 29
	50	96 ± 31	75 ± 21
	100	96 ± 36	90 ± 29
	200	102 ± 30	90 ± 27
	400	98 ± 19	86 ± 17
Gentamicin	5	51 ± 32[b]	34 ± 18
	10	49 ± 21[b]	39 ± 19
	20	51 ± 24[b]	45 ± 22
	40	51 ± 9[b]	45 ± 11

[a] Values are means ± standard deviation of 10 experiments.
[b] Values are statistically significantly different from controls at $P \leq 0.05$ (Student's t test).

significantly. These results are similar to those of Khan et al. (8), but differ from those of Forsgren and Schmeling, who found no inhibition of chemotaxis by gentamicin (4). Of the various beta-lactam drugs examined thus far, cefamandole was without effect on chemotaxis, opsonization, or phagocytosis of human PMN cells (13), whereas cefoxitin has an inhibitory effect on the chemotaxis of PMN cells (13). In our study, chemotaxis of cells was unaffected by preincubation with different concentrations of moxalactam.

Inhibition of chemotaxis by tetracyclines and gentamicin is mediated by the inactivation of human complement fraction 3 (1, 11). To also exclude this mechanism, moxalactam (25 to 50 µg/ml) was preincubated with serum, and the chemotactic capacity of that serum was not diminished. When we tested phagocytosis and killing capacity, neither gentamicin nor moxalactam interfered with those functions. The broad spectrum of in vitro activity of moxalactam, its low toxicity, and preliminary clinical results have made this drug a potentially useful agent for the treatment of severe infections in the compromised host. Even though the clinical significance of these laboratory findings is generally unknown, it seems desirable, especially when dealing with compromised hosts, to use antibiotics that do not interfere with natural host defense mechanisms (1, 3).

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