ACTION OF CEFOXITIN AND CEFAMANDOLE ON HUMAN NEUTROPHIL FUNCTION

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Polymorphonuclear neutrophilic leukocyte chemotaxis was examined *in vitro* in the presence of two new antibiotics: cefamandole and cefoxitin. Results indicate that cefamandole inhibited neutrophil chemotaxis to a significant degree only at high antibiotic concentrations of 100 μg/ml ($P<0.01$) and has no significant effect at normal serum therapeutic range. Cefoxitin was found to produce a 43% inhibition ($P<0.01$) of human *in vivo* neutrophil chemotaxis at antibiotic concentrations of 100 μg/ml and have a minimal inhibitory effect (1~9%) at low concentrations (1~5 μg/ml). Both cefamandole and cefoxitin had no significant effect on opsonophagocytosis.

Several promising antibiotics have been developed recently in the search for a cephalosporin that will combine satisfactory *in vitro* activity with relative resistance to hydrolysis by beta-lactamase. Two recently developed compounds, cefoxitin and cefamandole, have been reported to extend the antibacterial spectrum of the cephalosporin antibiotics[1,2]. Cefoxitin, although resembling cephalothin structurally, is actually a member of the cephamycin group of antibiotics[3]. Recent studies from this laboratory have reported the effects of various antibiotics on human neutrophil function[4] and chemotaxis[5], including an inhibitory effect by tetracycline[6]. This report is a continuation of these *in vitro* investigations on immune function under the influence of the newly developed antibiotics cefoxitin and cefamandole.

Material and Methods

Chemotaxis

Human leukocytes were obtained from healthy adult male donors between 25 and 35 years of age. No medications were taken by any donor for one month before neutrophil testing. Equal volumes of venous blood were added to a 6% dextran solution in normal saline (2,000 units of heparin/100 ml of dextran solution). The suspension was allowed to sediment at 37°C for 1 hour. The neutrophil rich (80~90%) supernatant was removed and centrifuged at 1,000 rev/min. in an International centrifuge (Model V) at room temperature. The pellet containing neutrophils (PMN) was resuspended and washed twice in GEY's solution (Flow Labs., Rockville, Md.) before use. Ninety-nine percent neutrophil viability was confirmed by the trypan blue exclusion method after sedimentation.

Disposable chemotactic chambers (Adaps, Inc., Dedham, Mass.) were used to study neutrophil chemotaxis across a nucleopore filter (Nucleopore Corporation, Pleasanton, Ca.) with a 3-μm pore size interposed between the upper and lower chambers. The membrane was attached to the upper chamber by partially dissolving the lower plastic edge with acetone and then fixing the membrane to the plastic surface. A one-ml aliquot of washed neutrophils (5×10^6 cells per ml) in GEY's solution was placed in the upper chamber. Chemotactic factors were generated from a mixture containing one part human serum and one part endotoxin solution (20 μg *Salmonella typhosa* 0901, Lipopolysaccharide B per ml; Difco, Detroit, Mich.) in GEY's solution. Type AB human serum from healthy donors was used as the serum source. Either sodium cefoxitin (Merck Sharp & Dohme, Rahway,

N.J.) or cefamandole lithium (Eli Lilly Co., Indianapolis, Ind.) was added to the upper and lower chambers at concentrations of 1, 5, 10, 50 and 100 μg per ml of solution. The antibiotics were supplied in powder form and were free of preservatives. Controls for the sodium and lithium salts were included with each experiment. The pH of the antibiotic solutions was altered to neutrality prior to experimental study. After the fluid-filled chambers were incubated for 2 hours, at 37°C, the filters were removed and stained using a direct drying technique previously described[7]. All the migrating cells in 10 high power fields (HPF) were counted on each filter. All assays were done in triplicate. The reported chemotactic activities are the averages of the counts obtained ± the standard deviation of the mean.

To assess whether antibiotics influenced random motion of neutrophils, PMNs from five subjects were isolated. The top and bottom compartments of triplicate experimental chambers were filled with 10 μg/ml, final concentration, of sodium cefoxitin or cefamandole lithium. In contrast to previous experiments, the top and bottom compartments of each chamber contained equal final amounts of chemotactic factors.

Test for opsonophagocytosis

Resistant strains of *Pseudomonas aeruginosa* immunotype 2 were made by incubating the organism in trypticase soy broth with the respective antibiotic at a concentration of 20 μg/ml. The resistant strains were centrifuged and washed twice in HANK's Gel Solution (HGS) and resuspended at a concentration of 5×10^7 organisms/ml. Human neutrophils were obtained from healthy donors, and the blood was aliquoted in equal volumes to tubes containing an equal volume of 6% dextran in normal saline and heparin (500 units/tube). The suspension was allowed to sediment at 25°C for 1hour. The neutrophil-rich supernatant was removed and centrifuged at $250\times g$ for 5 minutes at room temperature. After washing the cells twice in HGS (Flow Laboratories, Rockville, Md.), the neutrophils were adjusted to a concentration of 10^7 cells/ml with HGS.

The opsonizations were performed in 75×12 polypropylene tubes. Cefoxitin and cefamandole were tested at concentrations of 0, 1, 5, 10 and 20 μg/ml. For each concentration of antibiotic, four sample tubes were made. All tubes contained 4% pooled normal human serum. Two of the four tubes were incubated at 56°C for one-half hour to test for non-complement dependent opsonic and phagocytic activity. The two tubes not incubated tested both the classical and alternative pathways of complement activation. To one of each set of two tubes was added a 0.5-ml suspension of neutrophils (5×10^6 cells), while 0.5 ml of HGS was added to the other tube. After the appropriate amount of antibiotic was added, 0.1 ml of the bacterial suspension was added to each tube. The final volume per tube was 1 ml. The test samples were incubated for 3 hours, at 37°C on an aliquot mixer. Dilutions of 10^{-2}, 10^{-3}, and 10^{-4} of the test were made in sterile water and then plated on Trypticase soy agar plates. The plates were incubated for 48 hours at 37°C and the colonies counted, to determine the log of the surviving number of bacteria.

Results and Discussion

Both cefoxitin and cefamandole were found to inhibit the human *in vitro* neutrophil chemotactic response (Table 1.) Cefoxitin was found to produce an inhibition of 40% at concentrations of 100 μg/ml ($P<0.01$, *t*-test). In the clinical therapeutic range of serum antibiotic concentration, cefoxitin produced an inhibition of 22% at 10 μg/ml ($P<0.05$), and an inhibition of 9% at 5 μg/ml ($P<0.05$). Cefamandole significantly inhibited *in vitro* PMN chemotaxis only 10% at concentrations of 100 μg/ml ($P<0.01$). The slight inhibition of chemotaxis by cefamandole in the clinical therapeutic range of antibiotic concentrations was not statistically significant. No effect was demonstrated with the sodium or lithium salts on neutrophil chemotaxis.

The effect of cefoxitin and cefamandole on the random migration of PMN is shown in Table 2. No significant differences were found between cefoxitin or cefamandole and control cells. It therefore

Table 1. Effect of cefoxitin and cefamandole upon the chemotaxis of human neutrophils as observed in a chemotactic chamber.

Antibiotic	Concentration of antibiotic (μg/ml)	Chemotactic activity (PMN/10 HPF ±S.D.)*
Control		382±40
Control+sodium salt (10μg/ml)		371±29
Control+lithium salt (10μg/ml)		376±32
Cefoxitin	100	217±61
	50	241±49
	10	296±21
	5	351±44
	1	373±33
Cefamandole	100	345±42
	50	362±46
	10	373±14
	5	376±16
	1	380± 8

* Polymorphonuclear leukocytes per 10 high power fields ± standard deviation.
n=5.

Table 2. Effect of 10 μg of cefoxitin or cefamandole per ml on random migration of PMNs to non-gradient concentrations of chemotactic factor.

Antibiotic	Concentration of antibiotic (μg/ml)	Random migration (PMN/10 HPF ±S.D.)*
Control	—	29±8
Cefoxitin	10	29±8
Cefamandole	10	28±5

* Polymorphonuclear leukocytes per 10 high power fields ± standard deviation.
n=5.

appears that the slight *in vitro* inhibition by cefoxitin of PMNs exposed to a concentration gradient of chemotactic factors is a result of impaired, directed migration. The results from these experiments agree with previously reported data for the cephalosporin antibiotics. Cephalothin was found to inhibit *in vitro* chemotaxis only at large concentrations[5].

The results of the opsonization tests, shown in Table 3, indicate no significant effect of either cefoxitin or cefamandole impairing the complement dependent or non-complement dependent opsonic and phagocytic activity of serum.

The search for new antibiotics is directed toward the discovery of agents with desirable pharmacological properties, as well as greater activity against pathogenic bacteria but without any untoward effects on the host immune system. Cefamandole and cefoxitin are examples of these newer antibiotics. The *in vivo* spectrum of these two antibiotics indicates that cefamandole, with its increased spectrum and high intrinsic antibacterial activity, and cefoxitin, with its resistance to hydrolysis by beta-lactamase, both offer distinct advantages over other cephalosporins[8,9]. The inhibition of neutrophil chemotaxis by cefoxitin and cefamandole are minimal in the normal range of clinically attained *in vivo* serum levels. Patients with altered or depressed neutrophil function, such as DOWN's syndrome, CHEDIAK-HIGASHI syndrome and large thermal burns, should be administered specific antibiotics which do not alter or inhibit neutrophil activity or function. Tetracycline, as an example, has been shown to interfere with the bactericidal effect of serum, inhibit *in vitro* neutrophil chemotaxis in concentrations as low as 1 μg/ml and interfere with the activation of the alternate pathway of complement[6]. Though *in vitro* neutrophil function results cannot be directly compared to *in vivo* data, certain implications may be postulated. The implications of this study suggest that (a) cefamandole has no untoward effect on *in vitro* neutrophil chemotaxis and opsonophagocytosis and (b) cefoxitin is associated with a statistically significant impairment of the polymorphonuclear leukocyte chemotactic system. As more laboratory studies occur and basic data become available, correlations of these data will more readily be applicable with *in vivo* host immunity and the treatment of infection.

Table 3. Effect of cefoxitin and cefamandole on complement and non-complement dependent pathways of opsonophagocytosis.

	Antibiotic	Amount of antibiotic (μg/ml)	Log kill (Log of sample without cells minus log of sample with cells)	Opsonic index $\left(\frac{\text{log kill of test}}{\text{log kill of control}}\right)$
Normal serum	Cefoxitin	20	2.56	1.13
		10	2.25	0.98
		5	2.26	0.99
		1	2.25	0.98
		Control	2.29	—
	Cefamandole	20	2.51	0.98
		10	2.55	1.00
		5	2.80	1.10
		1	2.35	0.92
		Control	2.55	—
Heat inactivated serum	Cefoxitin	20	1.50	1.12
		10	1.60	0.99
		5	1.70	0.93
		1	1.93	0.87
		Control	1.72	—
	Cefamandole	20	1.02	0.53
		10	1.77	0.92
		5	1.76	0.92
		1	1.62	0.84
		Control	1.92	—

n=4

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