PROPHYLACTIC ACTIVITY OF CEPHALOSPORINS IN A MOUSE MODEL OF SURGICAL WOUND INFECTION

SARAH F. GRAPPEL*, LILLIAN PHILLIPS, HUGH B. LEWIS,
D. GWYN MORGAN and PAUL ACTOR

Smith Kline and French Laboratories, Philadelphia, PA 19101, U.S.A.

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A mouse model was developed which would simulate a surgical wound infection. The model consists of an infected foreign body granuloma which is induced by implanting, subcutaneously, a filter paper disk saturated with carrageenan and a suitable number of bacteria to initiate infection. The ability of cefonicid and cefamandole, administered one hour prior to implantation, to prevent establishment of infection with several bacterial species, including *Staphylococcus aureus* and *Escherichia coli*, was compared. A single 40 mg/kg dose of cefonicid administered subcutaneously prior to disk implantation protected against the establishment of the local infection, peritonitis and dissemination of the infecting organism to the systemic organs. A similar dose of cefamandole had no effect on the progress of the infections. The local as well as the systemic responses of the mice were characterized. Both the hematologic and the histopathologic pictures of the cefonicid-treated groups resembled those of the uninfected control groups. The response in groups treated with cefamandole resembled the untreated, infected controls. Both cefonicid and cefamandole penetrated into the implanted disk. However, only cefonicid could still be detected four hours after administration.

Cefonicid (SK&F 75073) is a parenteral broad-spectrum cephalosporin with high and prolonged blood levels in animals and man[1,2]. The efficacy of cefonicid as a prophylactic antibiotic for lethal bacterial infections in mice has already been demonstrated[3]. In order to assess further the potential usefulness of this cephalosporin as a prophylactic antibiotic for surgery, a mouse model was developed to simulate a surgical wound infection and the septicemia which results from spread of the infection. The efficacy of cefonicid as a prophylactic antibiotic was compared with that of cefamandole in this mouse model.

Materials and Methods

Antibiotics

Cefonicid (SK&F 75073) was synthesized at Smith Kline and French Laboratories. Cefamandole (Mandol) was a commercial preparation from Eli Lilly Laboratories. Each of the cephalosporins was dissolved in sterile saline. They were administered subcutaneously in a volume of 0.5 ml in the back of the neck at a dose of 40 mg/kg, 1 hour prior to implantation of the disks. Experiments were carried out with groups of 10 mice each as controls and for each compound tested.

Antimicrobial Activity *In Vitro*

The minimum inhibitory concentrations of cefonicid and cefamandole for the bacterial strains used were determined by microtiter two-fold dilution tests in Mueller-Hinton broth. An inoculum of approximately 10^5 cfu/ml was used. The tests were incubated at 37°C overnight.

Induction of Foreign Body Granuloma

Webster-derived CD-1 male mice, 27~28 g, were anesthetized by ether inhalation and secured on their backs. Skin of the ventral abdomen was incised in the midline and a 6.35-mm diameter filter paper disk saturated with a 1.0% sterile carrageenan solution was implanted in the underlying sub-

cutaneous tissue. The incision was closed with wound clips.

Induction of an Infected Foreign Body Granuloma

An appropriate dilution of a log phase Trypticase soy broth culture of each bacterial strain was mixed with an equal volume of a 2.0% sterile carrageenan solution. A filter paper disk was saturated with this bacterial suspension and implanted subcutaneously as described above. The inoculum size employed was the lowest which would produce a local infection in 100% of the mice.

Bacteriological Evaluation of Local and Systemic Infections

Mice were sacrificed on the fifth day after implantation of the disks (day 1). At this time, the disks were removed, observed grossly and placed in 9.0 ml of Trypticase soy broth to prepare a bacterial suspension for counting. Dilutions of the suspension were plated on Trypticase soy agar and incubated at 37°C overnight.

In addition, peripheral blood was obtained by cardiac puncture; and peritoneum and selected organs were removed aseptically for determination of the dissemination of organisms. Blood or organs were cultured on Trypticase soy or eosin methylene blue agar at 37°C.

Detection of Cephalosporin Penetration into the Implanted Disk

Cephalosporins, 40 mg/kg, were administered subcutaneously to mice immediately after implantation of disks saturated with 1.0% carrageenan. Groups of 10 mice were sacrificed at one and four hours after implantation, and the disks were removed and placed on Trypticase soy agar plates seeded with *Staphylococcus aureus* 209P or *Escherichia coli* KN. The plates were incubated at 30°C overnight, and the zones of growth inhibition were measured.

Pathology

Three male mice from each group, prepared surgically as described above, were examined histologically, cytologically and hematologically to assess the local and systemic responses to infection. The groups comprised the following treatments:

Group 1a and 1b — disk + carrageenan,
Group 2 — disk + carrageenan + *S. aureus* 674,
Group 3 — disk + carrageenan + *S. aureus* 674 + cefonicid,
Group 4 — disk + carrageenan + *S. aureus* 674 + cefamandole,
Group 5 — disk + carrageenan + cefonicid,
Group 6 — disk + carrageenan + cefamandole.

On the fifth day after disk implantation, the mice were anesthetized with CO_2 and killed by exsanguination.

Blood and Marrow Samples

1.0 ml of blood was taken from the posterior vena cava, into K_2EDTA. A total white blood cell count was done with a Coulter Counter Model S. A differential white blood cell count was done on blood smears stained with Wright's stain. Smears of bone marrow obtained from the left femur were prepared using a gentle brush technique, with a sable brush and autologous serum. These were also stained with Wright's stain.

Tissue Samples for Cytologic and Histologic Examination

The implantation site, including skin, subcutaneous tissue, filter paper disk and ventral abdominal musculature, was resected as a whole, and cut in half with a scalpel. One half was immediately fixed in 10% formol-saline. Imprint smears were prepared from the cut edge of the other half. In addition, the right femur with bone marrow, and the spleen from each mouse were fixed in 10% formol-saline. Cytologic preparations were stained with Wright's stain. Fixed tissues were processed routinely, sectioned at five microns and stained with hematoxylin and eosin.

Results

Antimicrobial Activity of Cefonicid and Cefamandole *In Vitro*

Each of the bacterial strains used to produce local infections in mice was susceptible *in vitro* both

to cefonicid and to cefamandole. The minimum inhibitory concentrations of these antibiotics are given in Table 1. For the Gram-positive strains employed, cefamandole was clearly more active. Cefonicid tended to show superior activity against the Gram-negative pathogens.

Table 1. Minimum inhibitory concentrations of cefonicid and cefamandole for strains used to establish wound infections.

Strain	MIC (μg/ml)	
	Cefamandole	Cefonicid
S. aureus tour 674	0.4	1.6
S. aureus 127	1.6	6.3
S. epidermidis 2479	0.2	3.1
E. coli 12140	0.4	0.4
P. mirabilis 442	0.8	$\leq$0.1
K. pneumoniae 4200	0.4	$\leq$0.1

Establishment of Infection *In Vivo*

The smallest inoculum size which resulted in a reproducible local infection with each strain *in vivo* was used in the assays. A purulent infection was established at the site of implantation of the disk. It was evaluated in terms of colony-forming units (CFU) per disk. Except for the *Klebsiella pneumoniae* infection, the inocula of the bacterial strains used did not result in death in the four day test.

Prophylactic Antimicrobial Activity of Cefonicid and Cefamandole *In Vivo*

Prophylactic administration of cefonicid protected against establishment of a local infection with all of the bacterial strains tested and protected against mortality caused by *K. pneumoniae* (Table 2). The local infections were equally well established in untreated infected controls and in those that received cefamandole. *K. pneumoniae* caused death in both these groups. Cefonicid completely prevented establishment of the local infection with the common Gram-negative bacterial strains tested. In contrast, the infections in the cefamandole-treated mice resembled those in the non-treated controls.

In spite of its (four-fold) higher minimum inhibitory concentration *in vitro* compared to cefamandole against the strains of *Staphylococcus* used, cefonicid either prevented the establishment of the infection or decreased the bacterial count from the implanted disk. The infections established locally with both

Table 2. Prophylactic activity of cefonicid and cefamandole against establishment of wound infections in mice[a].

Strain No., inoculum (cfu/disk)	cfu/disk in survivors				
	Control	Cefamandole		Cefonicid	
	Median (range)	Median (range)	% Negative	Median (range)	% Negative
S. aureus 674 (9×10^2)	10^7 ($10^7\sim10^8$)	10^6 ($10^6\sim10^7$)	0	Zero ($0\sim10^6$)	60
S. aureus 127 (1.8×10^5)	5×10^6 ($10^6\sim10^7$)	10^6 ($10^5\sim10^6$)	0	10^4 ($0\sim10^5$)	10
S. epidermidis 2479 (4.5×10^7)	10^5 ($10^4\sim10^6$)	10^4 ($0\sim10^6$)	10	Zero ($0\sim10^4$)	90
E. coli 12140 (8×10^5)	10^7 ($10^6\sim10^8$)	10^8 ($10^6\sim10^8$)	0	Zero only	100
P. mirabilis 442 (2×10^2)	10^7 ($10^6\sim10^8$)	10^7 ($10^6\sim10^8$)	0	Zero only	100
K. pneumoniae 4200[b] (1.8×10^5)	5×10^7 ($10^6\sim10^8$)	10^7 ($10^6\sim10^8$)	0	Zero only	100

[a] Compounds, 40 mg/kg, s.c., 1 hour prior to implantation of disk.
Infections evaluated on day 5 after implantation.
Zero = <90 cfu/disk.

[b] Non-treated controls, 8/10 survivors; cefamandole, 9/10 survivors; cefonicid, 10/10 survivors.

Table 3. Prophylactic activity of cefonicid and cefamandole in protection of mice against establishment of systemic infections.[a]

Infection			Detection of bacteria[b]							
Strain inoculum (cfu/disk)	Compounds	Number survivors	Disk	Peritoneal fluid	Peritoneum	Lungs	Blood	Spleen	Liver	Kidneys
E. coli 12140	Control	10 / 10	100	10	20	40	20	50	60	50
(1.5×10^5)	Cefonicid	10 / 10	0	0	0	0	0	0	0	0
	Cefamandole	10 / 10	100	10	10	40	40	60	50	40
S. aureus 674	Control	10 / 10	100	100	40	60	40	90	90	90
(4.5×10^3)	Cefonicid	10 / 10	100	100	50	50	10	60	60	60
	Cefamandole	10 / 10	100	100	80	90	40	90	80	80

[a] Compounds, 40 mg/kg s.c., 1 hour prior to disk implantation.
[b] Data given as percent positive.

S. aureus and *S. epidermidis* in mice treated with cefamandole resembled those in the untreated infected controls.

In most instances, the local infections became systemic (Table 3). Cefonicid, but not cefamandole, reduced the frequency with which *E. coli* 12140 was recovered from viscera. *S. aureus* 674 was recovered with almost the same frequency in all of the groups. However, the organs were not counted. There was a reduction in the cfu/disk at the local site where it was quantitated (Table 2).

Penetration of Cefonicid and Cefamandole into the Implanted Disk

Both cefonicid and cefamandole penetrated into the disk at the site of implantation early since there was detectable activity in the disks one hour after administration of each of the cephalosporins. Cefonicid persisted at detectable levels up to four hours (Table 4). Zones of inhibition of strains both of *E. coli* and of *S. aureus* were obtained with disks removed at this time. No zones of inhibition were observed with disks removed four hours after treatment with cefamandole.

Hematology

The total and differential white blood cell counts of mice sacrificed on day 5 after disk implantation are given in Table 5. Normal ranges for mice are also given in the Table. The values for uninoculated untreated mice (groups 1a and 1b) and those given cefonicid (group 5) or cefamandole (group 6) prior to implantation of an uninfected disk were similar and were within the normal range. Bone marrow smears from these mice were normal.

The untreated mice that received a disk inoculated with *S. aureus* 674 (group 2) showed a leukocytosis, marked neutrophilia and monocytosis. A bone marrow smear from these mice showed granulocytic

Table 4. Detection of cefonicid and cefamandole at site or disk implantation[a].

Hours post disk implantation	No. disks tested	Strain seeded on plates	No. disks positive	
			Cefonicid	Cefamandole
1	5	*E. coli* KN	5	0
4	5	*E. coli* KN	5	0
1	5	*S. aureus* 209P	5	5
4	10	*S. aureus* 209P	10	0

[a] Cephalosporins administered, s.c., 40 mg/kg, immediately post implantation of disks. Disks removed one and four hours post injection and placed on seeded agar plate. Positive=Zone of inhibition.

Table 5. Total and differential white blood cell count of mice.*

Group	Prophylaxis	WBC	Neutrophils	Lymphocytes	Monocytes
1a	None	11.3	3.1	7.4	0.6
1b	None	8.7	0.9	7.4	0.4
2	None	17.6	10.3	6.0	0.9
3	Cefonicid	5.7	0.6	4.9	0.1
4	Cefamandole	13.7	6.9	5.2	1.6
5	Cefonicid	10.0	1.4	8.4	0.1
6	Cefamandole	7.6	1.1	6.0	0.4
Normal range**		7.0~15.0	0.1~6.0	3.5~6.0	0.0~0.3

* Group mean values. Absolute number $\times 10^3/mm^3$. Cephalosporins were administered at 40 mg/kg, s.c. 1 hour prior to disk implantation. Infection initiated with *S. aureus* 674, groups 2, 3 and 4.

** Reference 4: SCHALM *et al.*, 1975.

hyperplasia and an increased number of immature granulocytes. Similar results were obtained with mice of group 4 which received cefamandole prior to implantation of a disk inoculated with *S. aureus* 674.

The white cell counts of mice receiving cefonicid prior to implantation of a disk inoculated with *S. aureus* 674 (group 3), however, were well within the normal range and were similar to those of control mice (groups 1a, 1b, 5 and 6). Bone marrow smears of group 3 mice were likewise normal.

Histologic and Cytologic Examination

The salient results are summarized in Table 6. There was minimal subcutaneous inflammatory reaction in untreated mice (groups 1a and 1b) and in those administered cefonicid (group 5) or cefamandole (group 6) prior to implantation of an uninoculated disk. Bone marrow and spleens of these mice were histologically normal.

A severe necrotizing, fibrinopurulent inflammation was seen at the site of implantation in untreated, infected mice (group 2) and in those given cefamandole (group 4) prior to implantation of an *S. aureus*

Table 6. Histologic and cytologic examination of tissues.

Group	Prophylaxis*	Site of disk implantation	Bone marrow	Spleen	Disk imprint
1a 1b 5 6	None None Cefonicid Cefamandole	Slight inflammatory cell infiltration, polymorph and macrophages	Normal hematopoietic activity	Normal extramedullary hematopoiesis	Moderate numbers of erythrocytes and leukocytes; few active macrophages
2 4	None Cefamandole	Severe fibrinopurulent inflammation; bacterial colonies in disks	Reactive granulocytic hyperplasia	Congested; granulocytic hyperplasia	Purulent exudate, degenerate neutrophils, cell debris and many bacteria
3	Cefonicid	Slight inflammatory cell infiltration	Normal hematopoietic activity	Normal extramedullary hematopoiesis	Few erythrocytes and leukocytes; no bacteria; slightly reactive macrophages

* Cephalosporins were administered at 40 mg/kg, s.c. 1 hour prior to disk implantation. *S. aureus* 674 was used to initiate infection, groups 2, 3 and 4.

inoculated disk. Colonies of Gram-positive bacteria were seen histologically within the disk and many similar bacteria were present in a cytologic imprint of the implantation site. Both the spleen and bone marrow of these mice showed evidence of granulocytic hyperplasia.

In contrast, mice given cefonicid prophylactically (group 3) showed minimal tissue reaction to implantation of a *S. aureus* inoculated disk. This local reaction was similar to that seen in mice implanted with a carrageenan disk (groups 1a, 1b) and was commensurate with a mild response to a foreign body. The histologic appearance of bone marrow and spleen was normal.

Discussion

Several models of surgical wound infections have been described in the literature[5]. Although some, such as the infected incision wounds, may serve as useful predictors of therapeutic efficacy, even these models have shortcomings.

The model described here was developed in order to simulate a surgical wound infection in the mouse, the most convenient laboratory animal for evaluating antimicrobial agents. It provides a tool for predicting the utility of compounds for surgical prophylaxis. The local infection is established with a relatively small number of bacteria, requires small amounts of drug for testing and is readily evaluated by several criteria as it runs its course. In addition, the use of filter paper disks in uninfected treated mice allows for measurement of drug penetration into the implantation site.

The superiority of cefonicid over cefamandole and other commercially available cephalosporins as prophylactic agents for Gram-positive and Gram-negative lethal infections in mice was reported earlier[3]. The superior activity *in vivo* of cefonicid over cefamandole given prior to infection was confirmed in this model by using the most common pathogens, including *S. aureus*. There was a correlation between the bacteriological evaluations and the pathologic data. Both the local and the systemic inflammatory responses to the infection were minimized or eliminated by prophylactic administration of cefonicid but were not affected by cefamandole. The high and prolonged serum levels achieved with cefonicid and its ability to penetrate tissues may account for its excellent activity *in vivo*[1,6]. The antibacterial action of cefonicid fully accounted for the observed moderation in the inflammatory response. There was no evidence of any anti-inflammatory activity *per se* due to cefonicid.

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