ACTIVITY OF CEFTIZOXIME AND COMPARATIVE COMPOUNDS AGAINST *BACTEROIDES FRAGILIS* IN A MOUSE MODEL OF ANAEROBIC INFECTION

SARAH F. GRAPPEL*, LILLIAN PHILLIPS and PAUL ACTOR

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

(Received for publication November 14, 1983)

A new mouse model of anaerobic infection with *Bacteroides fragilis* alone or in a mixed infection with *Escherichia coli* is described. It is established by implantation under the skin of a filter paper disk saturated with the appropriate bacterial suspension. The penetration of antibiotics into the implantation site can be detected by assaying the disk. The local infection can be both standardized and evaluated by determining the bacterial count on the disk. The antimicrobial efficacy of ceftizoxime was compared with other commercially available antibiotics administered in a single dose, 40 mg/kg subcutaneously, one hour after implantation of the disk. Using such a regimen ceftizoxime was found to be superior to a clindamycin-gentamicin combination and equal to or superior to cefoxitin in these models.

The possibility of developing models of infection in mice with *Bacteroides fragilis,* both alone and in combination with *Escherichia coli* was explored. A new method has recently been used in our laboratories to produce local infections with aerobic bacteria in mice[1]. These infections are easily evaluated and respond to appropriate antibiotic therapy. This methodology was adapted to produce the anaerobic models described in this study. Ceftizoxime is a broad spectrum cephalosporin with activity against many strains of *B. fragilis*[2]. The therapeutic activity of this cephalosporin was compared with cefoxitin and a clindamycin-gentamicin combination in these experimental infections.

Materials and Methods

Infection Model

B. fragilis ATCC 25285 and *E. coli* 12140 were used to establish infections. Both *B. fragilis* and *E. coli* were grown in chopped meat glucose broth, Scott Laboratories, under anaerobic conditions at 37°C for 18 hours. A mixed infection with these organisms was established both in normal and in cyclophosphamide-treated mice. An infection with *B. fragilis* alone was established consistently only in cyclophosphamide-treated mice. Cyclophosphamide was administered ip at a dose of 300 mg/kg, four days prior to infection to produce neutropenia (Table 1).

For the mixed infection in normal mice, a suspension consisting of equal parts of the *B. fragilis* culture and a 10^{-1} dilution of the *E. coli* culture was mixed with an equal volume of 2.0% sterile carrageenan. Disks were saturated with the suspension. For the mixed infection in cyclophosphamide-pretreated mice, equal parts of a suspension consisting of equal parts of an undiluted *B. fragilis* culture and a 10^{-4} dilution of an *E. coli* culture and 2.0% carrageenan were mixed. For the *B. fragilis* infection, equal parts of an undiluted culture and 2.0% carrageenan were mixed. 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 filter paper disk saturated with the inoculum in 1.0% sterile carrageenan (Viscarin, Marine Colloids, Division of FMC Corporation, Springfield, N.J., U.S.A.) solution was implanted in the underlying subcutaneous tissue. The incision was closed with a wound clip. Mice were held for six additional days (day 7) following implantation of the disk.

They were sacrificed on day 7, the areas surrounding the disks were observed and the disks were removed, suspended and diluted in Schaedler's broth and the dilutions counted to determine the number of bacteria, colony forming units (cfu) per disk. The counts were done on Schaedler's agar in an anaerobic chamber for *B. fragilis* and on Trypticase soy agar, aerobically for *E. coli*. The plates were incubated at 37°C. Groups of 10 mice were used for each antibiotic tested and for non-treated infected controls.

Drugs

Cyclophosphamide was a commercial preparation from Mead Johnson. Cefoxitin was an investigational sample from Merck, Rahway, N. J. Clindamycin and gentamicin were commercial preparations. Ceftizoxime was a research sample from Fujisawa, Osaka, Japan. All of the antimicrobial agents except gentamicin were administered at 40 mg/kg, sc. Gentamicin was used at 1.0 mg/kg, sc. The compounds were given as a single dose, one hour post implantation.

Detection of Antibiotics in the Implanted Disk

Groups of 15 mice per antibiotic were injected subcutaneously immediately after implantation of a 1.0% carrageenan-saturated disk. The mice were sacrificed one hour after administration of the antibiotics. The disks were removed and five from each group were placed on agar plates seeded with *Staphylococcus aureus* 209P, *E. coli* KN, and *B. fragilis* ATCC 25285. The plates were incubated overnight at 37°C and zones of inhibition were recorded.

Results

Pretreatment of mice with cyclophosphamide allowed establishment of a consistent infection with *B. fragilis* impregnated into a disk and implanted under the skin. There were no deaths in these mice at day 7. A consistent infection with *B. fragilis* could also be established if a mixed inoculum consisting of *E. coli* and *B. fragilis* was used in the normal mouse. Early death occurred in this infection probably due to the *E. coli*. When *E. coli* was eliminated by treatment, there was no death even though *B. fragilis* sometimes persisted. A smaller *E. coli* inoculum was required to produce death in the mixed infection in neutropenic mice (Table 1). In all of the infections, the disks in the surviving control-untreated mice were covered with purulent exudate and contained viable *B. fragilis*.

The *in vitro* activities of each of the antibiotics against the *B. fragilis* and *E. coli* strains used in these infections are given in Table 2. At low inoculum levels, *B. fragilis* was sensitive to each of the antibiotics except gentamicin. There was a marked decrease in the activity of ceftizoxime at the higher inoculum used. *E. coli* was sensitive to each of the antibiotics except clindamycin.

The therapeutic efficacies of a single dose of ceftizoxime and comparative compounds in these infections are given in Table 3. In the mixed infections, ceftizoxime protected against death and

Table 1. Establishment of infections with *Bacteroides fragilis* in mice.

Infection	Mouse	Inoculum	
		Strains	Approximate cfu/disk[a]
Mixed	Normal	*Escherichia coli* 12140	1×10^6
		Bacteroides fragilis ATCC 25285	3×10^7
Mixed	Neutropenic[b]	*E. coli* 12140	3×10^3
		B. fragilis ATCC 25285	1.5×10^7
B. fragilis	Neutropenic[b]	*B. fragilis* ATCC 25285	3×10^7

[a] Estimate based on 30 μl of inoculum per disk.
[b] Cyclophosphamide, 300 mg/kg, ip, 4 days prior to infection.

decreased the counts or completely eliminated both *E. coli* and *B. fragilis,* both in the normal and in the neutropenic mouse. Many of the disks were clear of exudate. In the *B. fragilis* infection in the neutropenic mouse, ceftizoxime reduced the median count per disk. The disks in these mice were not covered with as much exudate as the controls and some were clear. In contrast, with the limited regimen used, the clindamycin-gentamicin combination did not completely prevent death in the normal mice and did not protect the neutropenic mice significantly. The counts of *E. coli* were not reduced and the *B. fragilis* counts were higher than in the group treated with ceftizoxime. In general, the site surrounding the disk implant looked more purulent and necrotic in most of these mice than in the untreated control infections. In the *B. fragilis* infection in the neutropenic mice, the median count per disk for the clindamycin-gentamicin group was the same as in the ceftizoxime group. Although one out of 9 disks was negative for *B. fragilis,* the site surrounding the disk implant in the remaining mice had more purulent exudate than those in the control, untreated infections.

Table 2. Minimum inhibitory concentrations (MIC) of ceftizoxime and comparative compounds against *B. fragilis*[a] and *E. coli*.[b]

Compound	MIC (μg/ml)		
	B. fragilis ATCC 25285		*E. coli* 12140
	High	Low	
Ceftizoxime	100.0	6.3	0.4
Cefoxitin	12.5	12.5	6.3
Clindamycin	0.63	0.31	100.0
Gentamicin	100.0	100.0	3.1

a) Anaerobic determinations: Wilkins-Chalgren agar, 37°C, 48 hours, Capco anaerobic chamber. Inoculum: *B. fragilis*, High=approximately 10^8 cfu; Low=approximately 10^4 cfu.

b) Aerobic determinations: Cation supplemented Mueller-Hinton broth, 37°C overnight. Inoculum: *E. coli*, approximately 10^5 cfu/ml.

Cefoxitin protected against death and cleared or reduced the counts of both *E. coli* and *B. fragilis* in the normal mouse. However, it was less effective than ceftizoxime in the neutropenic mouse both in preventing death and in decreasing the counts of bacteria per disk. The disks were heavily covered with exudate. In the *B. fragilis* infection in the neutropenic mouse, the site of the disk was covered with purulent exudate in all of the animals. There was less of a reduction in the median count per

Table 3. Activity of ceftizoxime and comparative compounds in mouse infections.[a]

Compound	Mixed infection, normal mouse			Mixed infection, neutropenic mouse			*B. fragilis* infection, neutropenic mouse	
	Survivors	*E. coli* (cfu/disk)	*B. fragilis* (cfu/disk)	Survivors	*E. coli* (cfu/disk)	*B. fragilis* (cfu/disk)	Survivors	*B. fragilis* (cfu/disk)
Control (no treatment)	7/10	10^6 $10^6 \sim 10^8$	10^7 $10^6 \sim 10^8$	1/10	10^8	$>10^8$	10/10[b]	10^7 $10^5 \sim 10^8$
Clindamycin-gentamicin	9/10	10^7 $10^5 \sim 10^8$	10^6 $<10^2 \sim 10^8$ 30% Neg.	2/10	10^8 Only	$<10^2$	10/10[b]	10^5 $<10^2 \sim 10^7$ 10% Neg.
Ceftizoxime	10/10	5×10^3 $10^2 \sim 10^6$ 50% Neg.	5×10^4 $<10^2 \sim 10^7$ 50% Neg.	10/10	5×10^3 $<10^2 \sim 10^6$ 50% Neg.	10^6 $<10^2 \sim 10^8$ 10% Neg.	10/10	10^5 $10^4 \sim 10^7$
Cefoxitin	10/10	$<10^2$ $<10^2 \sim 10^3$ 80% Neg.	5×10^4 $<10^2 \sim 10^8$ 50% Neg.	9/10	10^8 $10^4 \sim 10^8$	10^7 $10^5 \sim 10^8$	10/10	10^8 $10^4 \sim 10^7$

a) Compounds administered, sc, 1 hour post-implantation, 40 mg/kg except gentamicin, 1 mg/kg. Cfu/disk expressed as median and range of counts. Percent of disks with no counts given. $<10^2$=no bacteria counted. Neg.=negative. Infections evaluated on day 7 post disk implantation.

b) Nine of ten disks counted.

disk than with ceftizoxime.

Ceftizoxime, as well as cefoxitin and the clindamycin-gentamicin combination, could be detected in disks removed from the site of implantation one hour after subcutaneous administration of the antibiotics (Table 4).

Table 4. Detection of antibiotics in disk from site of implantation.[a)]

Compound	No. disks tested	Seeded plates		
		B. fragilis ATCC 25285	*S. aureus* 209P	*E. coli* KN
Ceftizoxime	5	+	+	++
Cefoxitin	5	−	+	−
Clindamycin	5	+	+	+

[a)] Median zone diameter; ++, >18 mm; +, ≤18 mm; −, no zone.

Ceftizoxime, clindamycin, gentamicin and cefoxitin disk levels were sufficient to be detected by activity on plates seeded with *E. coli* and *Staphylococcus aureus*. However, only ceftizoxime and clindamycin levels were sufficient to be detected by activity against the *B. fragilis* strain used.

Discussion

Several animal models of anaerobic infection have been previously described and reviewed in the literature. Establishment of *B. fragilis* infection usually requires the use of mucin or agar or synergy with other microorganisms[3~8]. In order to predict antimicrobial efficacy against *B. fragilis, in vivo,* the most commonly used model of intra-abdominal sepsis is produced in Wistar rats challenged ip with pooled colonic contents in gelatin capsules[9,10,11]. The resulting infection is biphasic, initially there is acute peritonitis, *E. coli* bacteremia and mortality. The survivors then develop intra-abdominal abscesses in which anaerobes predominate. Challenge ip with *E. coli* alone in gelatin capsules produces bacteremia while challenge ip with *B. fragilis* alone in gelatin capsules produces abscesses.

A model of local infection with *B. fragilis* alone and in combination with *E. coli* was developed to test for *in vivo* efficacy of antimicrobial agents against *B. fragilis*. The mouse models of anaerobic infection described here can be easily standardized to give reproducible infections. Establishment of the infection by use of an implanted filter paper disk also allows detection of the penetration of antibiotics to the site of infection and ease in estimating the number of bacteria at the local site. These infections allow assessment of therapeutic activity of antimicrobial agents against *B. fragilis* alone as well as in a model of polymicrobic infection which is more analogous to the naturally acquired infections in man and to the course of infection in the rat model commonly used to predict efficacy against anaerobes[11]. In these mouse infections, death also occurs early due to *E. coli* while *B. fragilis* alone does not result in fatal infection.

In these mouse models, infection with *B. fragilis* could only be established consistently if the mice were made neutropenic or if a mixed infection was established with *E. coli,* either in normal or in neutropenic mice. These findings are consistent with the observations of previous investigators who required mucin or agar to establish *B. fragilis* and those who have demonstrated the need for bacterial synergy with other microorganisms in order to produce *B. fragilis* infections.

The efficacy obtained with ceftizoxime and comparative compounds in this mouse model is in agreement with the results obtained by others in the commonly accepted Wistar rat model[12].

Acknowledgment

We thank Mr. ROBERT REID for his excellent technical assistance.

References

1) GRAPPEL, S. F.; L. PHILLIPS, H. B. LEWIS, D. G. MORGAN & P. ACTOR: Prophylactic activity of cephalosporins in a mouse model of surgical wound infection. J. Antibiotics 36: 161~166, 1983
2) FU, K. P. & H. C. NEU: Antibacterial activity of ceftizoxime, a β-lactamase-stable cephalosporin. Antimicrob. Agents Chemother. 17: 583~590, 1980

3) KELLY, M. J.: The quantitative and histological demonstration of pathogenic synergy between *Escherichia coli* and *Bacteroides fragilis* in guinea pig wounds. J. Med. Microbiol. 11: 513~523, 1978
4) KELLY, M. J.: Wound infection: A controlled clinical and experimental demonstration of synergy between aerobic (*Escherichia coli*) and anaerobic (*Bacteroides fragilis*) bacteria. Annals of the Royal College of Surgeons of England 62: 52~59, 1980
5) ONDERDONK, A. B.; D. L. KASPER, B. J. MANSHEIM, T. J. LOUIE, S. L. GORBACH & J. G. BARTLETT: Experimental animal models for anaerobic infections. Rev. Infect. Dis. 1: 291~301, 1979
6) INGHAM, H. R.; P. R. SISSON & J. B. SELKON: Current concepts of the pathogenetic mechanisms of nonsporing anaerobes; Chemotherapeutic implications. J. Antimicrob. Chemother. 6: 173~179, 1980
7) WILKINS, T. D.; C. B. WALKER, D. NITZAN & A. A. SALYERS: Experimental infections with anaerobic bacteria in mice. J. Infect. Dis. 135: S13~S17, 1977
8) JOINER, K. A.; A. B. ONDERDONK, J. A. GELFAND, J. G. BARTLETT & S. L. GORBACH: A quantitative model for subcutaneous abscess formation in mice. Brit. J. Exp. Pathol. 61: 97~106, 1980
9) WEINSTEIN, W. M.; A. B. ONDERDONK, J. G. BARTLETT & S. L. GORBACH: Experimental intra-abdominal abscesses in rats: Development of an experimental model. Infect. Immun. 19: 1250~1255, 1974
10) ONDERDONK, A. B.; W. M. WEINSTEIN, N. M. SULLIVAN, J. G. BARTLETT & S. L. GORBACH: Experimental intra-abdominal abscesses in rats: Quantitative bacteriology of infected animals. Infect. Immun. 10: 1256~1259, 1974
11) BARTLETT, J. G.; A. B. ONDERDONK, T. LOUIE, D. L. KASPER & S. L. GORBACH: A review: Lessons from an animal model of intra-abdominal sepsis. Arch. Surgery 113: 853~857, 1978
12) LOUIE, T. J.; G. K. M. HARDING, H. BELL & J. KOSS: Ceftizoxime therapy of experimental intra-abdominal sepsis. *In* Abstracts of the 21st Intersci. Conf. on Antimicrob. Agents & Chemother., No. 548, Chicago, 1981