LABORATORY STUDIES WITH CEFATRIZINE (SK&F 60771) A NEW BROAD-SPECTRUM ORALLY-ACTIVE CEPHALOSPORIN

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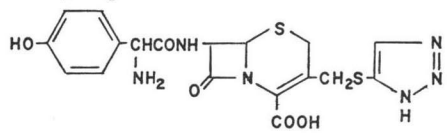
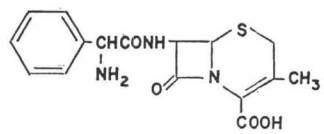
Cefatrizine (SK&F 60771), a new orally-active semisynthetic cephalosporin antibiotic with broad-spectrum antibacterial activity, was compared with cephalexin and cefazolin for *in vitro* and *in vivo* antibacterial activity and pharmacokinetic behavior in laboratory animals. The average MIC values obtained with cefatrizine against gram-positive and gram-negative bacteria were superior to those obtained with cephalexin and somewhat poorer than those of cefazolin. In addition, a large percentage of the enterobacter and enterococcus isolates were found to be susceptible. Cefatrizine had a longer biological half-life and a higher peak serum level than either cefazolin or cephalexin when administered parenterally or orally to mice at 20 mg/kg. It had striking *in vivo* protective activity in mice infected with *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Hemophilus influenzae*, *Proteus morganii* or *Staphylococcus aureus* reflecting its superior pharmacokinetic profile in this animal species. A variable pharmacokinetic response between animal species was observed when cefatrizine was administered either orally or parenterally to dogs, squirrel monkeys or rabbits.

Cefatrizine (SK&F 60771), a new cephalosporin antibiotic, is one of a series of 3-heterocyclic thiomethylcephalosporins first synthesized in our laboratories[1]. Reports describing the microbiological activity and pharmacokinetic properties in rodents have been published by Bristol Laboratories, whose code number is BL-S 640[2,3,4]. Chemically, cefatrizine is 7-[R(−)-2-amino-2-(*p*-hydroxyphenyl)acetamido-]-3-(1H-1, 2, 3-triazole-4 (5)-ylthiomethyl)3-cephem-4-carboxylic acid (Fig. 1). The present report details our findings with this cephalosporin comparing it with cephalexin and cefazolin, commercially available antibiotics for oral and parenteral use, respectively.

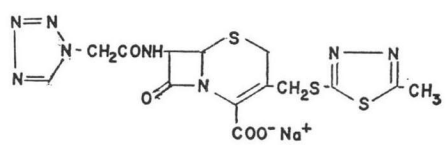
Fig. 1. Chemical structures of cefatrizine (SK&F 60771), cephalexin and cefazolin



Cefatrizine (SK&F 60771)



Cephalexin



Cefazolin

Materials and Methods

Cephalosporins

The structures of the cephalosporins employed in these studies are shown in Fig. 1. Cefatrizine (SK&F 60771) and cefazolin were prepared in the Smith Kline & French Laboratories and cephalexin was obtained from Eli Lilly and Company.

Efficacy Studies

Male albino Swiss-Webster mice weighing 18~20 g were used for the efficacy studies. Cultures of the test organisms were diluted in either trypticase soy broth (*Klebsiella*) or 5 % hog gastric mucin and injected intraperitoneally to produce uniformly lethal mouse infections. Unless noted otherwise, test drugs were dissolved in isotonic sodium chloride solution and administered either orally or subcutaneously at 1 and 5 hours after infection. Final survival percentages for groups of 10 mice each, obtained after observation for 3 days, were used to estimate the ED_{50} (total mg/kg of antibiotic required to protect 50 % of the infected animals) and the LD_{50} values (dose of inoculum required to kill 50 % of the mice). The ED_{50} and LD_{50} values were determined by the method of LITCHFIELD and WILCOXON[5]. The minimum inhibitory concentrations (MIC) were determined by the agar dilution method as described previously[6]. The MIC values were determined on nutrient agar except for *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *N. meningitidis* and *H. influenzae* where the test medium was MUELLER-HINTON Agar fortified with IsoVitalex and Fildes enrichments and buffered with HEPES to pH 6.8.

Microbiological Assay

Disc-plate assays with *Bacillus subtilis* ATCC 6633 as the indicator organism were employed to determine serum and urine concentrations of the antibiotics. For serum assays, standard curves were prepared by plotting dose-response data for each antibiotic using pooled sera as diluent for the standard. For urine assays, standard curves were prepared in 0.01 N phosphate buffer (pH 6.0). Assay plates were incubated for 18 hours at 30°C. Zone diameters were measured with a Fisher-Lilly Zone Reader.

Pharmacokinetic Studies

Serum levels in laboratory animals were determined at selected time intervals after oral or parenteral administration. The cephalosporins were dissolved in water using 5.7 % bicarbonate solution, if necessary, and adminstered orally or parenterally at a level of 20 mg/kg. Oral administration was via intubation except for dogs where the cephalosporin was administered in a gelatin capsule. Parenteral administration was via the intramuscular route except for mice who were dosed subcutaneously. Blood samples were obtained from mice by decapitation of duplicate pooled groups of 10 mice each. Sequential blood samples were drawn from the jugular vein in the dog, the femoral vein in the squirrel monkey, and the marginal ear vein in the rabbit. Blood samples were kept at 4°C until clotting occurred and the serum obtained after centrifugation was stored at −20°C prior to assay. The serum half-life after parenteral or oral administration was estimated from a semi-log plot of the antibiotic levels *versus* time. A cross-over protocol was employed for all the studies involving dogs, squirrel monkeys and rabbits. Urinary antibiotic recovery during the four-hour period after dosing was determined in duplicate groups of ten mice. The mice were housed in metabolism cages and the urine was collected in bottles packed in dry ice. Dog urine samples were collected at 0~6 and 6~24 hours after administration. Dogs were catheterized at 6 hours to clear the bladder of unvoided urine. Voided urine was collected from metabolism pans into cold plastic containers. All urine samples were stored at −20°C prior to assay.

Results

In Vitro Studies

The minimum inhibitory concentrations obtained with cefatrizine, cephalexin and cefazolin against 174 bacterial isolates representing 10 genera are shown in Table 1. In general, the *in vitro* potency of cefatrizine was 4 to 8-fold better than that observed with cephalexin. When compared with cefazolin, cefatrizine tended to be somewhat less potent; however, against selected groups of organisms such as enterococci and enterobacter, it demonstrated superior activity. This was especially true against enterobacter species where the median MIC for cefatrizine was

Table 1. Minimum inhibitory concentrations of cefatrizine (SK&F 60771), cephalexin and cefazolin against bacterial isolates as determined by agar dilution in nutrient agar

Organism	No. Isolates	Median minimum inhibitory concentration (mcg/ml)		
		SK&F 60771	Cephalexin	Cefazolin
S. aureus	15*	0.8	3.1	0.2
Enterococcus	14	12.5	50.0	18.8
E. coli	30	3.1	12.5	1.6
K. pneumoniae	15	3.1	25.0	1.6
P. mirabilis	21	3.1	25.0	3.1
Proteus (Indole positive)	23	50.0	200.0	50.0
Enterobacter sp.	15	6.3	50.0	50.0
Providencia	7	50.0	25.0	25.0
S. marcescens	29	>200.0	>200.0	>200.0
Citrobacter	5	25.0	200.0	50.0

* 11 of the 15 isolates were resistant to penicillin G.

6.3 mcg/ml, whereas those of cephalexin and cefazolin were 50 mcg/ml. In data not shown in this table, the median MIC for cefatrizine against 23 strains of *S. pneumoniae* was 0.4 mcg/ml, for 55 *N. gonorrhoeae* isolates 3.2 mcg/ml, and for 23 *N. meningitidis* isolates 0.4 mcg/ml. Approximately 90 % of *Hemophilus* strains were inhibited at levels of 6.3~12.5 mcg/ml. Cefatrizine was not active against *S. marcescens*, and *Pseudomonas*. The activity against indole-producing proteus strains was not of a high order, however, approximately 42 % of the strains were inhibited at antibiotic concentrations of 25 mcg/ml or less (Fig. 3). Figs. 2 and 3 plot the cumulative percent of the strains inhibited against the MIC values for seven of the more important genera of bacteria isolated from clinical specimens. These plots for cefatrizine, cephalexin and cefazolin clearly point up differences between the three antibiotics. Against all seven species, the poorest activity by far was observed with cephalexin. With the exception of enterobacter and enterococci, the intrinsic activity of cefazolin tended to be superior to that of cefatrizine; cefazolin was more active against strains of *E. coli*, *K. pneumoniae*, *P. mirabilis* and *S. aureus*. The enterobacter strains showed a wide variation in susceptibility to cefatrizine, however, this

Fig. 2. Activity of cefatrizine (SK&F 60771), cephalexin and cefazolin against isolates of *K. pneumoniae*, *E. coli* and *Enterobacter*

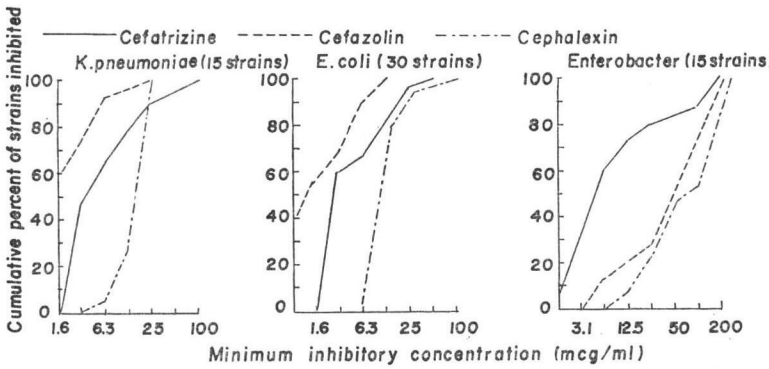
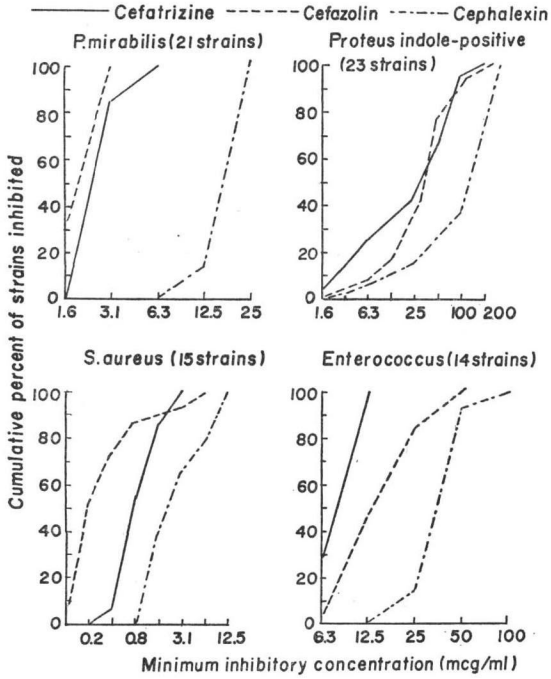


Fig. 3. Activity of cefatrizine (SK&F 60771), cephalexin and cefazolin against isolates of indole-negative *Proteus*, indole-positive *Proteus*, *S. aureus* and enterococcus



antibiotic was clearly superior to cephalexin and cefazolin against this bacterial genus. The enterococcus strains were also markedly more susceptible to cefatrizine than to the other two antibiotics.

In Vivo Studies

The efficacy of cefatrizine was compared with that of cephalexin and cefazolin in mice. The results obtained along with the corresponding MIC values for 5 genera of bacteria are shown in Table 2. Cefatrizine is compared orally with cephalexin and subcutaneously with cefazolin. Although the MIC values of cefatrizine were two to four times better than cephalexin's against *E. coli*, *K. pneumoniae* and *S. aureus*, the *in vivo* efficacy was four to fifty times greater. It is of interest that the oral ED_{50} of cefatrizine against *P. morganii* was 19 mg/kg even though the MIC value was 50 mcg/ml. The subcutaneous ED_{50} values of cefatrizine were markedly superior to cefazolin's despite the fact that its MIC values against the test organisms tended to be poorer. Table 3 lists the data obtained with the three cephalosporins in mice against four strains of *E. cloacae* and one strain of *E. aerogenes*.

Table 2. *In vitro* and *in vivo* antibacterial activity of cefatrizine (SK&F 60771), cephalexin and cefazolin

Test organism	Strain No.	Test No.	MIC (mcg/ml)			ED_{50} (mg/kg)				Challenge inoculum (LD_{50})
						SK&F 60771		Cephalexin	Cefazolin	
			SK&F 60771	Cephalexin	Cefazolin	S.C.	P.O.	P.O.	S.C.	
E. coli	12140	1	1.6	6.3	1.6	<0.8	<0.8	14	5	210
		2				0.8	10.0	14	4	1000
		3				0.5	0.5	18	4	333
K. pneumoniae	4200	1	3.1	6.3	1.6	0.6	0.4	21	25	83
		2				0.6	0.5	17	16	13
S. aureus (penicillin resistant)	127	1	1.6	3.1	0.4	3	3	11	3	25
H. influenzae	ATCC A9006	1	6	NT	7	2	3	25	11	500
P. morganii	179	1	50	>200	>200	44	19	>200	>200	200

NT—Not Tested

Table 3. *In vitro* and *in vivo* activity of cefatrizine (SK&F 60771), cephalexin and cefazolin against strains of enterobacter

Enterobacter	Strain No.	MIC (mcg/ml)			ED_{50} (mg/kg)				Challenge inoculum (LD_{50})
					SK&F60771		Cephalexin	Cefazolin	
		60771	Cephalexin	Cefazolin	S.C.	P.O.	P.O.	S.C.	
E. cloacae	928	6.3	12.5	6.3	84	>200	>200	86	17
E. cloacae	922	3.1	25.0	100	1.0	1.0	93	>200	166
E. cloacae	924	3.1	25.0	100	1.0	1.0	132	>200	30
E. cloacae	891	3.1	25.0	25	>200	>200	>200	>200	>1000
E. aerogenes	510	6.3	12.5	3.1	>200	>200	>200	>200	25

Table 4. Effect of challenge inoculum size on activity in an *E. coli* 12140 infection in mice

Cephalosporin	ED_{50} (mg/kg)		Challenge inoculum (LD_{50})
	S.C.	P.O.	
SK&F 60771	0.5	1.0	1×10^2
Cefazolin	6.0	NT	
Cephalexin	NT	35.0	
SK&F 60772	1.5	2.0	1×10^3
Cefazolin	>200	NT	
Cephalexin	NT	>200	

NT—Not Tested

Table 5. Effect of a single dose of cefatrizine (SK&F 60771), cephalexin or cefazolin administered to mice prior to challenge with *E. coli* 12140

Test #	Dosage time (hrs)	ED_{50} (mg/kg)			
		SK&F 60771		Cephalexin	Cefazolin
		S.C.	P.O.	P.O.	S.C.
1*	−1/2	0.1	0.3	>25	>25
	−2	0.5	0.5	>25	>25
	−5	0.7	0.4	>25	>25
2*	−5	6.0	3.0	>200	>200
	−8	>25	12.5	>200	>200
	−18	>100	>100	>400	>400

* LD_{50} of 50 for test No. 1 and 100 for test No. 2

Table 6. Effect of time of dosage on activity (ED_{50}, mg/kg) of cefatrizine (SK&F 60771), cephalexin and cefazolin after *E. coli* 12140 infection in mice*

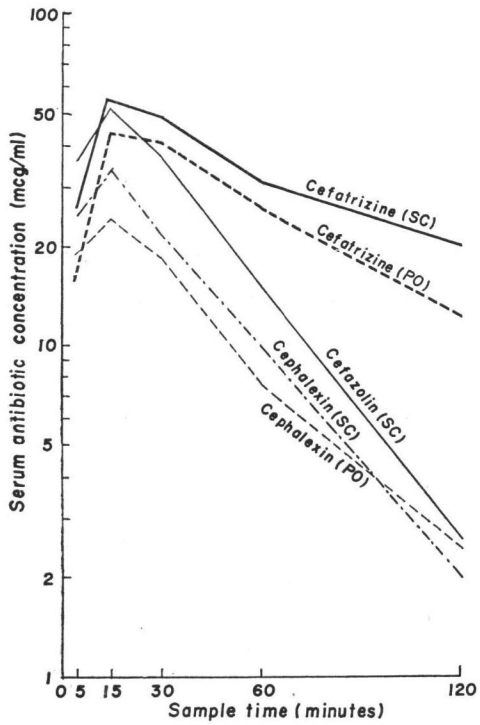
Cephalosporin	Dosage time (Hours) after infection					
	1 Hour		5 Hour		1 and 5 Hour	
	S.C.	P.O.	S.C.	P.O.	S.C.	P.O.
SK&F 60771	0.9	0.6	0.9	0.9	0.8	1.0
Cephalexin	NT	>25	NT	>25	NT	25
Cefazolin	>25	NT	>25	NT	7.4	NT

* Challenge Inoculum Size: 1×10^3 LD_{50}
NT—Not Tested

Excellent protective activity with cefatrizine was obtained after oral or subcutaneous administration against two of the *E. cloacae* strains (#922, #924), whereas, the other two antibiotics showed poor or no activity.

The effect of inoculum size on the activity of these three cephalosporins against an *E. coli* infection in mice is shown in Table 4. When the challenge inoculum was increased from 10^2 to 10^6 LD_{50}, cefatrizine continued to show excellent protective activity, whereas, cefazolin and cephalexin failed to protect even at 200 mg/kg. Tables 5 and 6 show the effect of a single dose of the cephalosporins administered at various time intervals prior to or after challenge with *E. coli* in mice. Excellent protective activity was observed with a single dose of cefatrizine adminstered up to 5 hours before infection, whereas cephalexin and cefazolin gave no protection at the levels tested (Table 5). Similar results were obtained when the three antibiotics were administered in a single dose up to 5 hours after infection (Table 6).

Fig. 4. Antibiotic serum concentrations in mice dosed orally and subcutaneously with cefatrizine (SK&F 60771), cephalexin and cefazolin



Serum Levels and Urinary Recovery in Laboratory Animals

The antibiotic serum concentrations of the three cephalosporins following subcutaneous or oral administration at 20 mg/kg to mice are shown in Fig. 4. It can be seen from the figure that administration of cefatrizine to mice resulted in high and sustained serum levels. At two hours after oral or subcutaneous administration of cefatrizine, the antibiotic serum concentrations were 12.5 mcg/ml and 20 mcg/ml, respectively, whereas those of cefazolin and cephalexin were less than 3 mcg/ml. The peak serum concentrations and biological half-life of the three cephalosporins in four animal species are shown in Tables 7 and 8. Following oral administration to mice, the peak antibiotic concentration and serum half-life of cefatrizine was approximately twice that of cephalexin (Table 7). In rabbits the pharmacokinetic profiles of cefatrizine and cephalexin after oral dosing were similar. In

Table 7. Peak serum antibiotic concentrations and half-lives in laboratory animals following oral administration of cefatrizine (SK&F 60771) or cephalexin at 20 mg/kg

Species	Cefatrizine (SK&F 60771)		Cephalexin	
	Peak (mcg/ml) concentration	$t\frac{1}{2}$ (minutes)	Peak (mcg/ml) concentration	$t\frac{1}{2}$ (minutes)
Mouse	44	62	24	36
Dog[a]	16	80	30	98
Squirrel monkey[a]	2.8	120	13	66
Rabbit[a]	12	93	14	102

[a] Crossover protocol

Table 8. Peak serum antibiotic concentrations and half-lives in laboratory animals following parenteral administration of cefatrizine (SK&F 60771) or cefazolin at 20 mg/kg

Species	Cefatrizine (SK&F 60771)		Cefazolin	
	Peak (mcg/ml) concentration	$t\frac{1}{2}$ (minutes)	Peak (mcg/ml) concentration	$t\frac{1}{2}$ (minutes)
Mouse	54	71	52	23
Dog[a,b]	13	95	22	54
Squirrel monkey[a]	36	44	74	41
Rabbit[a]	48	24	68	27

[a] Crossover protocol—i.m. administration
[b] 10 mg/kg

dogs and squirrel monkeys, cephalexin's peak serum concentration exceeded that of cefatrizine. In mice, the urinary recovery of cefatrizine at four hours after oral administration was 24 % as compared with 55 % for cephalexin and 50 % for cefazolin. In the dog, urinary recovery of cefatrizine was 20 % at 24 hours post oral administration and 72 % for cephalexin.

When cefatrizine was compared to cefazolin following parenteral administration, a similar mixed response was observed in the four animal species examined (Table 8). As was found for the oral administration, parenteral administration resulted in an enhanced pharmacokinetic profile in the mouse, whereas the other animal species showed a more variable response. The urinary recovery for cefatrizine in mice (23 %) and dogs (18 %) was found to be lower than that of the control cefazolin (50 % in mice, 68 % in dogs).

Discussion

Cefatrizine (SK&F 60771) was found to have potent antibacterial activity *in vitro* against a broad spectum of clinical isolates. The activity observed was two to eight fold better than that of cephalexin and approximately one-half that of cefazolin. Against enterobacter and enterococcus, cefatrizine was clearly more potent than either cephalexin or cefazolin. In mice this new cephalosporin is absorbed rapidly after oral administration and peak serum level (44 mcg/ml) is approximately twice that of cephalexin (24 mcg/ml). In addition, its half-life is markedly prolonged resulting in effective serum antibiotic concentrations (12.5 mcg/ml at 2 hours) after oral administration. Similar high and extended serum levels were obtained in mice after subcutaneous administration. This improved pharmacokinetic profile is in part responsible for the excellent protective activity observed after oral or subcutaneous administration to infected mice. Administration of cefatrizine even five hours prior to lethal challenge with *E. coli* resulted in excellent protection. In untreated control animals infected with *E. coli*, death is usually observed within 18 hours after infection. Despite this rapid course of infection, a single oral or subcutaneous dose of cefatrizine given at five hours post-infection protected the animals (ED_{50} 0.9 mg/kg). Under the severe conditions of this test, cephalexin and cefazolin were more than 25 times less active. Cefatrizine was effective against massive challenge with *E. coli* (10^6 LD_{50} administered IP), whereas, the other two cephalosporins failed to protect mice under the same conditions.

The serum levels obtained after oral or parenteral adminstration of cefatrizine were found to vary in different animal species. Although the data obtained in mice show elevated and sustained antibiotic serum levels with oral or parenteral cefatrizine, this was not always the case in the other animal species. In the rabbit the peak serum levels and half-lives were similar to cephalexin after oral administration and to cefazolin's after intramuscular administration. Oral administration of cefatrizine to squirrel monkeys resulted in low and prolonged serum levels, whereas the serum levels obtained with cephalexin were significantly higher. The wide variation in response in these animal species is not surprising as other cephalosporins have been shown to produce a mixed response in different animal species[7].

From the laboratory studies presented here, it is evident that SK&F 60771 is an unusual and interesting cephalosporin deserving further investigation.

Acknowledgements

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