A NEW PARENTERAL CEPHALOSPORIN. SK&F 59962: *IN VITRO* AND *IN VIVO* ANTIBACTERIAL ACTIVITY AND SERUM LEVELS IN EXPERIMENTAL ANIMALS

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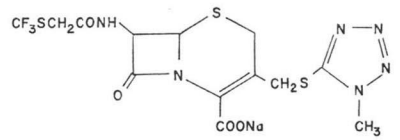
SK&F 59962, a new parenteral cephalosporin was found to have a high order of *in vitro* and *in vivo* antibacterial activity against a broad-spectrum of clinical isolates. When tested *in vitro* against gram-negative organisms, SK&F 59962 was consistently more active than cefazolin and far superior to cephalothin. This new antibiotic had activity equal to that of cephalothin against gram-positive bacteria. Enterobacter species were found to be susceptible to SK&F 59962. In mouse infection studies using bacterial pathogens, SK&F 59962 had protective activity of the order of that of cefazolin and superior to that of cephalothin. Following parenteral administration the serum profile of SK&F 59962 in the mouse, dog and squirrel monkey was similar to that of cephalothin. SK&F 59962 and cephalothin had lower peak serum concentrations and shorter biologic half-lives than those of cefazolin.

In the preceeding paper a new series of cephalosporins having 7-acyl substituents derived from methylthioacetic acid and its sulfoxide and sulfone is described.[1] The most active member of this series was found to be a 7-trifluoromethylthioacetyl derivative designated as SK&F 59962 (Fig. 1). Initial observations on antimicrobial activity and serum levels in experimental animals with SK&F 59962 were presented previously[2,3,4] and are now reported in detail.

Fig. 1. Structure of SK&F 59962. 7-Trifluoromethylthioacetamido-3-(1-methyl-1H-tetrazol-5-ylthiomethyl)-3-cephem-4-carboxylic acid, sodium salt.



SK&F 59962

Materials and Methods

In Vitro Studies: The minimum inhibitory concentrations (MIC) were determined by the agar dilution method previously described.[5] Unless otherwise indicated, the assays were carried out on Trypticase Soy Agar (TSA) containing 0.5 % glucose and buffered to pH 6.0. For the organisms so indicated in Table 1, the test medium was MUELLER-HINTON Agar (MHA) fortified with IsoVitalex and Fildes enrichments and buffered with HEPES to pH 6.8. In studies in which the effects of culture media on antibiotic activity were examined, unaltered commercial media were employed. Appropriate dilutions of bacterial suspensions were applied in duplicate onto the agar with the aid of a STEERS' inocula replicating apparatus.[6] Antibiotic concentrations ranged from 0.1 to 200 μg/ml in two-fold dilutions. All plates were incubated for 18 hours at 37°C and the minimum quantity of compound capable of inhibiting growth (MIC) was determined. The bacterial isolates were obtained from hospital patients in various geographical locations in the United States and are part of our culture collection.

Mouse Protection Studies: The cephalosporins were tested for protective activity in male

mice weighing 18~21 g. Diluted cultures containing the appropriate pathogen in hog gastric mucin were injected intraperitoneally to produce uniformly lethal infections. The cephalosporins were administered subcutaneously at 1 and 5 hours after infection as previously described.[7] Four-fold dilutions of each antibiotic were administered to five groups of ten mice each. Survivors were observed for three days and the PD_{50} values were calculated.[8]

Serum Level Studies: Serum levels were determined at selected time intervals after parenteral administration of the cephalosporins in mice, squirrel monkeys and dogs[7]. The antibiotics were injected at 20 mg/kg into mice and squirrel monkeys and 10 mg/kg into dogs. Mice were dosed subcutaneously and squirrel monkeys and dogs intramuscularly. Sequential blood samples were obtained at selected time intervals from dogs and monkeys. Mouse blood samples for each time interval were obtained by decapitation of duplicate pooled groups of ten mice each. Serum levels were determined by disc-plate assay with *Bacillus subtilis* ATCC 6633 as the indicator organism.

Results

SK&F 59962 was found to have broad-spectrum *in vitro* antibacterial activity. The MIC values in comparison with those of cephalothin and cefazolin are listed in Table 1. Against gram-positive bacteria the activity of SK&F 59962 was approximately equal to that of cephalothin, while against susceptible gram-negative bacteria, SK&F 59962 showed activity superior to that of cephalothin (MIC values were 2~16 fold lower). In comparison with cefazolin, the MIC values were two to four times lower. As was found with the control cephalosporins, activity against enterococci, indole-positive proteus, serratia and pseudomonas strains was not of a high order. Fig. 2 compares the cumulative percent of the organisms inhibited by SK&F 59962, cefazolin and cephalothin versus the MIC values for four genera of common clinical isolates. As can be seen from this figure, SK&F 59962 showed excellent activity against these important genera of clinical isolates. At a level of 6.3 μg/ml of SK&F 59962, 100 % of the staphylococcal, klebsiella and proteus isolates were inhibited and 85 % of the *Escherichia coli* isolates.

For each of the three cephalosporins the level of activity was found to undergo little variation when tested in several culture media. This is shown in Fig. 3 where these cephalosporins are compared using 15 staphylococcal strains on four culture media: Trypticase Soy Agar

Table 1. Median MIC values of SK&F 59962, cephalothin and cefazolin against clinical isolates.

Organism	No. of Isolates	Median MIC (mcg/ml)		
		SK&F 59962	Cephalothin	Cefazolin
Staphylococcus aureus	44	0.2	0.2	0.4
*Streptococcus pyogenes**	15	0.15	0.1	0.15
*Streptococcus pneumoniae**	15	0.1	0.15	0.1
Streptococcus faecalis	14	50.0	25.0	25.0
*Neisseria meningitidis**	15	0.4	0.8	0.8
*Neisseria gonorrhoeae**	15	1.6	3.1	3.1
Escherichia coli	30	0.8	6.3	1.6
Klebsiella pneumoniae	15	0.8	6.3	1.6
Enterobacter sp.	29	1.6	25.0	6.3
Proteus mirabilis	21	3.1	6.3	6.3
Proteus sp. (indole-positive)	15	100.0	>200.0	50.0
Serratia marcescens	12	100.0	>200.0	>200.0

* Enriched MUELLER-HINTON agar.

Fig. 2. Activity of SK&F 59962, cephalothin and cefazolin against clinical isolates (30 strains of *E. coli*, 15 of *K. pneumoniae*, 44 of *S. aureus* and 21 of *P. mirabilis*)

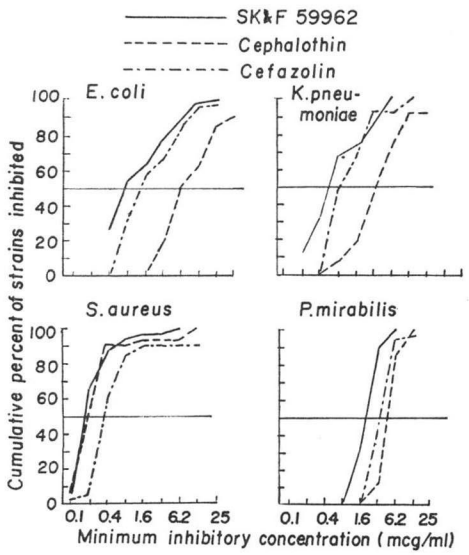


Fig. 3. Effect of culture medium on activity of SK&F 59962, cephalothin and cefazolin against fifteen staphylococcal strains.

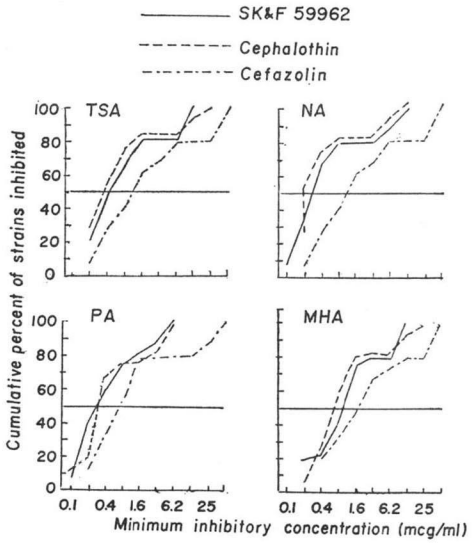
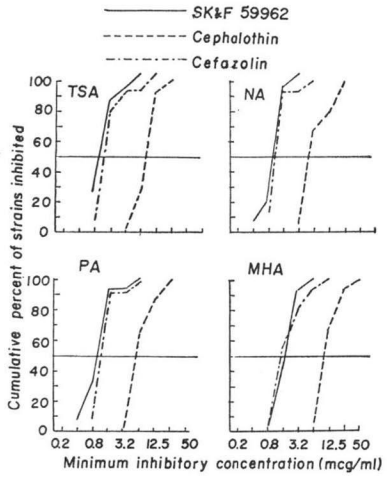


Fig. 4. Effect of culture medium on activity of SK&F 59962, cephalothin and cefazolin against fifteen *E. coli* strains.



(TSA), Nutrient Agar (NA), Penassay Seed Agar (PA) and MUELLER-HINTON Agar (MHA). In general, the MIC values for cefazolin were about 2-fold dilution higher than those of the other two cephalosporins. On MHA the MIC values for the three antibiotics were found to be 2- to 4-fold higher than those observed with the other media; however, the relative activities of the three antibiotics were similar in all of the culture media. The organisms in this study were both penicillin-sensitive and penicillin-resistant staphylococci including three methicillin-resistant strains. A similar study was carried out using 15 *E. coli* isolates (Fig. 4). On all four media, cephalothin showed decidedly less activity against these *E. coli* isolates than SK&F 59962 or cefazolin. Again, the three cephalosporins were less active on MHA.

One of the more striking differences in activity between SK&F 59962 and the two commercially available parenteral cephalosporins was observed against enterobacter species (Fig. 5). SK&F 59962 and cefazolin were compared using 15 enterobacter strains (three *E. aerogenes*, ten *E. cloacae*, two *E. hafniae*) on two culture media, TSA and MHA. Cephalothin data are not included since no significant activity was observed with this antibiotic against enterobacter. Although both SK&F 59962 and cefazolin showed good antimicrobial activity against the enterobacter strains in TSA, the significance of this finding is open to question since cefazolin is not

Table 2. Summary of infection-protection experiments in mice with SK&F 59962, cefazolin and cephalothin.

Test organism	Strain #	Challenge LD_{50}	PD_{50} (mg/kg)		
			59962	Cefazolin	Cephalothin
S. aureus	HH127	9	1.1	0.9	0.8
S. aureus	HH127	6	4.6	1.8	9.5
S. aureus	HH127	>1,000	10.2	8.7	>50.0
E. coli	12140	100	1.6	3.0	50.0
E. coli	12140	>1,000	9.5	11.0	—
E. coli	33779	>1,000	12.5	11.2	180.0
K. pneumoniae	4200	85	14.5	7.2	73.0
K. pneumoniae	1200	>1,000	1.8	5.2	38.0
Sal. paratyphi	ATCC 12176	2	5.5	6.3	43.0
E. cloacae	HH31254	42	5.0	10.2	>200.0
E. cloacae	HH31254	>1,000	80.0	37.0	>200.0
H. influenzae	ATCC A9006	13	2.3	10.0	5.1

Fig. 5. Effect of culture medium on the activity of SK&F 59962 and cefazolin against fifteen enterobacter strains.

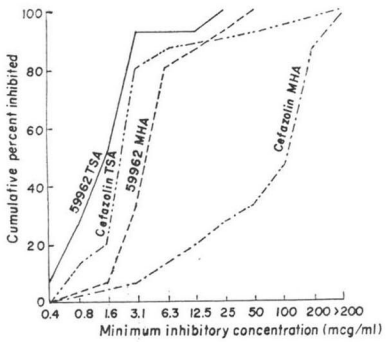
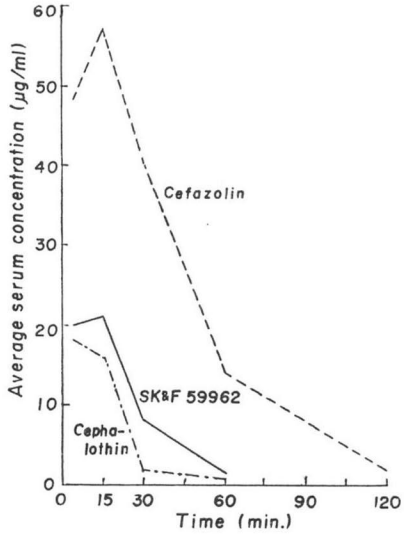


Fig. 6. Serum levels in mice of SK&F 59962, cephalothin and cefazolin after subcutaneous administration of 20 mg/kg.



generally considered to be of clinical use for the treatment of enterobacter (especially *E. cloacae*) infections. On MHA cefazolin had activity decidedly poorer than on TSA (this is more in line with its clinical use) while SK&F 59962 continued to demonstrate good activity on MHA against the enterobacter species.

Using several bacterial strains pathogenic to mice, SK&F 59962 was compared with cefazolin and cephalothin in a series of mouse infection-protection studies (Table 2). Organisms used included strains of *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi*, *E. cloacae* and *Haemophilus influenzae*. SK&F 59962 gave excellent protection when administered subcutaneously to mice infected against the gram-positive and gram-negative organisms, even when a heavy inoculum was used as the infecting dose. The protection afforded by SK&F 59962 was comparable to that of cefazolin's against gram-positive and gram-negative bacteria, while its activity was consistently superior to that of cephalothin against gram-negative pathogens, especially when a heavy inoculum was employed.

Fig. 7. Serum levels in squirrel monkeys of SK&F 59962, cephalothin and cefazolin after intramuscular administration of 20 mg/kg.

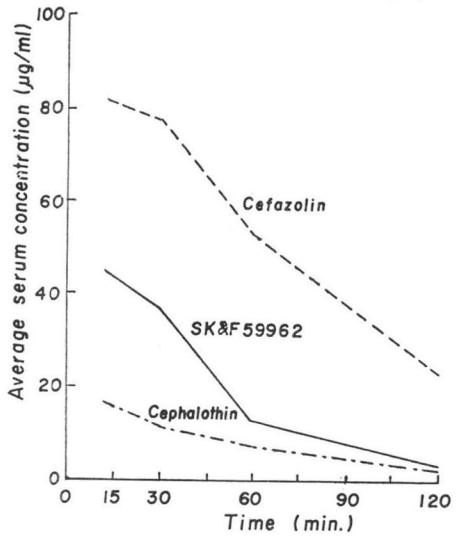
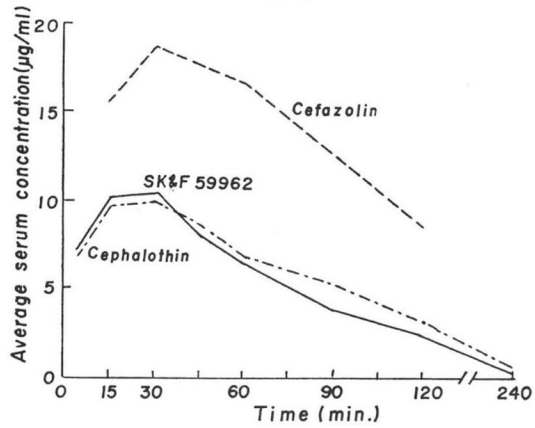


Fig. 8. Serum levels in dogs of SK&F 59962, cephalothin and cefazolin after intramuscular administration of 10 mg/kg.



Serum levels of SK&F 59962, cefazolin and cephalothin were determined at selected time intervals after parenteral administration to mice, squirrel monkeys and dogs. The average serum concentrations obtained in mice dosed subcutaneously at 20 mg/kg are plotted against time in Fig. 6. The serum levels obtained with SK&F 59962 in mice are comparable to those obtained with cephalothin. The cefazolin serum levels were clearly higher and more prolonged than those of the other two cephalosporins. Similar results were seen in squirrel monkeys (20 mg/kg) and dogs (10 mg/kg) after intramuscular administration (Figs. 7, 8). In these species also, cefazolin produced higher serum levels than SK&F 59962 and cephalothin.

Discussion

The *in vitro* and *in vivo* antibacterial activities and serum level profiles of SK&F 59962 were found to compare favorably with two commercially-available parenteral cephalosporins, cephalothin and cefazolin. In our *in vitro* test systems SK&F 59962 has been found to be one of the most potent cephalosporins evaluated in our laboratory. Its *in vitro* spectrum also includes activity against enterobacter species, especially *E. cloacae*. Although the serum levels in experimental animals after parenteral administration were comparable to cephalothin's, the *in vivo* protective activity was far superior to that of cephalothin. This finding reflects the superior intrinsic activity of SK&F 59962 against the test organisms. Other factors which account for the excellent *in vitro* and *in vivo* activities observed may be related to its high order of chemical and metabolic stability that we have observed under a variety of laboratory conditions. Preliminary studies with SK&F 59962 show it to be bound to human serum protein to the same degree as cephalothin.

Further work with this highly promising new cephalosporin antibiotic is being carried out to determine it potential for use in man.

Acknowledgements

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